DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

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Via e-mail: foia@americanoversight.org

July 15, 2024

Austin Evers
American Oversight
1030 15th Street, B255
Washington, DC 20005

Re: FOIA Case No. 53778

Dear Mr. Evers:

This responds to your March 18, 2020, Freedom of Information Act (FOIA) request addressed to the FOIA Office, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH). Department of Health and Human Services' (HHS) policy calls for the fullest possible disclosure provided by the FOIA, 5 U.S.C. §552, consistent with the protections contained therein. The implementing HHS Regulations establish the criteria pursuant to which the FOIA is administered, *see* 45 C.F.R. Part 5. Copies of the FOIA and the HHS FOIA Regulations are located at: http://www.nih.gov/icd/od/foia/efoia.htm and http://www.nih.gov/icd/od/foia/cfr45.htm.

You requested:

1. All email communications (including email messages, complete email chains, email attachments, calendar invitations) sent by Director Anthony Fauci, containing any of the key terms listed below:
    a. Bossert
    b. Ziemer
    c. Borio
    d. Luciana
    e. Bolton
    f. Waddell
    g. NSC
    h. "National Security Council"
    i. Pandemic
    j. "global health"
    k. "health security"
    l. "bio defense"
    m. biodefense
    n. "bio-defense"
    o. Flu

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p. Influenza

American Oversight has limited its request to sent messages to reduce the volume of potentially responsive records. American Oversight still requests complete email chains. So, for example, if a government official sent a response to an incoming message containing one of the key terms above, the email chain containing the initially received message and the response is responsive to this request.

2. All email communications (including email messages, complete email chains, email attachments, calendar invitations) between (a) Director Anthony Fauci, and (b) any of the then-Executive Office of the President officials listed below:

i. John Bolton, former National Security Advisor
ii. Tom Bossert, former Homeland Security Advisor
iii. Rear Adm. Timothy Ziemer, former Senior Director for Global Health Security and Biodefense
iv. Luciana Borio, former National Security Council Director for Medical and Biodefense Preparedness
v. Any other officials communicating with an email address ending in @nsc.eop.gov

For items 1 and 2, please provide all responsive records from April 9, 2018, through May 17, 2018

NIAID searched their files and found 2,087 pages of responsive records, of which 53 pages contain equities belonging to other offices. Because NIH does not have jurisdiction over these records, **we are referring these pages for their review and direct response to you.**

The information being withheld is protected from release pursuant to Exemptions 4, 5, and 6 of the FOIA, 5 U.S.C. § 552 (b)(4), (b)(5) and (b)(6); and sections 5.31(d), (e) and (f) of the HHS FOIA Regulations, 45 CFR Part 5. Exemption 4 protects from disclosure trade secrets and commercial or financial information that is privileged and confidential. Exemption 5 permits the withholding of internal government records which are predecisional and contain staff advice, opinion, and recommendations. This exemption is intended to preserve free and candid internal dialogue leading to decision-making. Exemption 6 exempts from disclosure records the release of which would cause a clearly unwarranted invasion of personal privacy.

Should you wish to contact the offices, please direct your inquiry to the following:

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In certain circumstances provisions of the FOIA and HHS FOIA Regulations allow us to recover part of the cost of responding to your request. There are no charges associated with the NIH processing of your request. HHS FOIA will contact you if there are any fees associated with processing your request.

Sincerely,

Gorka Garcia-malene -S Digitally signed by Gorka Garcia-malene -S Date: 2024.07.15 10:37:59 -04'00'

Gorka Garcia-Malene
NIH FOIA Office, NIH

Enclosures: 2,034 pages (one PDF)

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More recently, sustained scale-up of private provider engagement has taken place in several key countries (Figure 1.4.1). Bangladesh and Myanmar have sustained a moderate level of private provider engagement for the past five years, with private notifications reaching 14%-18% of incident cases. Recently, India, Pakistan, and the Philippines all increased their engagement of private providers, with private notifications increasing to 9%-13% of incident cases in 2016. Unfortunately, Indonesia and Nigeria–two countries with substantial numbers of "missing" TB cases–have made little progress, with private notifications averaging just 4% and 1% of estimated incidence, respectively.

In Bangladesh, Myanmar, and Pakistan, engagement of large numbers of private primary care providers has been led by strong non-governmental organizations (NGOs) acting as intermediaries between providers and the NTPs. These mission-driven NGOs have identified enhancing private provider engagement for TB as part of their long-term role and have succeeded in attracting resources from multiple donors to sustain their work. Some are generalist NGOs, such as BRAC in Bangladesh and Mercy Corps in Pakistan; others are more focused on TB, such as Damien Foundation in Bangladesh and more recently Interactive Research and Development in Pakistan; Greenstar in Pakistan and Population Services International in Myanmar are social marketing organizations that have long engaged private markets for family planning and other health issues. All these organizations have in common an understanding of private providers, the ability to operate at scale, strong management systems (for human resources, information, and logistics), dynamic leadership, an aptitude for adaptation and innovation, and success in fundraising.

Efforts in Indonesia and the Philippines have focused on private specialists and hospitals rather than primary care providers. The NTPs have partnered with specialist-led associations (such as the Indonesia Pulmonologist Society and the Philippines Tuberculosis Society). However, much of the initial care-seeking and TB treatment in these countries are among private primary care providers, and therefore more effort to engage these providers will be needed. Social health insurance schemes, approaching full population coverage in both countries, are contracting with an increasing number of private providers for primary care services. Yet collaboration between the NTP and social health insurance remains quite limited.[15]

One of the most exciting developments is the recent political commitment in India to scale-up private provider engagement nationwide, building on the success of several large demonstration projects (Panel 1.4.1).[16] India's National Strategic Plan for TB (2017-2020) commits to a massive expansion of private

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provider engagement and calls for a six-fold increase in private notifications to 2 million patients per year by 2020, which would represent 75% of estimated TB incidence. If India's plan succeeds, it will be the first major high-burden country with a dominant private healthcare sector to align its TB program with the care-seeking patterns of its population. Private notification targets for Bangladesh, Pakistan, Indonesia, and the Philippines are much more modest: 18-24% of estimated TB incidence by 2020 (Figure 1). Overall, at least 10 countries have recently prepared PPM Action Plans,[17] and the latest round of GFATM funding (2018-2020) includes substantial components for private provider engagement in several countries.

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As successful experiences on private provider engagement accumulate, defined packages of interventions could be disseminated as templates that could be adapted for rapid scale-up.[18] The core interventions in such templates include defined activities to engage private providers (including stakeholder consultation, provider mapping and prioritization, relationship management, facilitating reporting of TB cases and data, and patient support for adherence), addressing financial and non-financial incentives for private providers, and ensuring private patients have access to quality drugs and diagnostics according to national protocols. While intervention packages can and have been summarized in general terms, continued innovation and adaptation should be encouraged.

In addition, legal and regulatory frameworks should be in place to ensure TB notification and quality services by private providers. Several countries have re-issued laws and regulations requiring providers to report cases, sometimes conditioning re-licensing and accreditation to TB notification.[19] While regulatory penalties may have a role to play, countries most successful in engaging private providers have invested more in enablers (such as call centers to facilitate notification) and incentives (such as easy access to drugs and diagnostics) while respecting private providers' interests. Professional societies can be and have been successfully engaged to help define best practices for TB among private providers.

Looking ahead, new opportunities and developments could enhance private provider engagement for TB in the coming years. First, success in a country like India could set an example that inspires other countries. Second, the digital revolution is finally reaching TB (Panel C). The use of information and communication technology (ICT) systems, coupled with call centers, can facilitate the engagement of private providers and provide digital, case-based information on private TB patients. Third, such ICT systems can enable additional innovations that further facilitate private provider engagement at scale, such as digital vouchers for drugs and diagnostics, adherence monitoring technologies, and digital

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payment of incentives and enablers to both patients and providers. Fourth, access to new and improved diagnostic and treatment tools, such as digital chest x-rays and Xpert MTB/RIF®, increased the value to private providers of engaging with the public sector. Finally, the emergence of social health insurance schemes for UHC offers an unprecedented platform to engage private providers at scale across all health conditions, and provides an opportunity to drive the quality of TB care in the private primary sector in countries like Indonesia and Philippines.[20,21]

1.4.5 Modeling the impact of optimal private sector engagement

Because of ~~T~~the burden of TB that is managed in the private sector globally, the impact of improving private sector engagement warrants consideration. Modeling commissioned for this report assessed how greater private sector engagement in a high-burden country~~,~~ like India, where private providers offer extended capability, could influence TB incidence and mortality~~.~~. In such a setting, strategies to improve quality of private sector care, such as subsidized TB diagnostics, and NTP-funded adherence support mechanisms for patients accessing care privately, would avert 21% of TB deaths over the next 30 years, saving around 8 million lives from TB [Figure 1.4.2]. Furthermore an estimate of the cost of these interventions~~,~~ suggests that they would involve an annual increase of US$290million in NTP costs. While this strategy alone would be insufficient to end the epidemic in India, it has the potential to substantially reduce the public health threat posed by TB. Further, enhanced private sector engagement in concert with other strategies to close gaps in the care cascade, such as targetted ACF interventions, optimization of diagnostic networks and improved adherence support strategies could lead to signifcant reductions in TB mortality over the next ~~thirty~~30 years (ref Arinaminapthy et al, in press, 2018).

1.4.4 Recommendations

Engaging private providers on a scale equal to their importance in primary healthcare will require a transformation of TB programs: new partnerships, modern data systems, new payment mechanisms, new skills, and different attitudes. Rather than simply acknowledge the need to "engage all providers," NTPs in countries with significant private healthcare sectors need to prioritize their engagement. Evidence of prioritization can be gauged by staffing, data collection, partnerships, work plans, performance measurement, and, above all, budgets. In their absence, end TB goals will not be achieved.

For high-burden countries with large private healthcare sectors, three critical actions must be taken to prioritize private provider engagement.

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- NTPs should set high-profile targets for engaging private providers and notifying private patients. NTPs and WHO should adopt a set of indicators that can be used to guage progress and effectiveness.
- NTPs and major funders of TB control should substantially increase funding to engage private for-profit providers. Countries should allocate funding for private providers commensurate with the importance of the private healthcare sector in their country. NTPs and funders should collect and disseminate data on the amount and proportion of funds dedicated to engaging private for-profit and non-profit providers, calculated using a consistent approach.
- NTPs should develop long-term partnerships with organizations, such as NGOs, that can engage private providers at scale on their behalf.
- Ministries of Health should develop systems of strategic purchasing that link the incomes of public and private providers to their performance in delivering TB services while protecting patients from financial ruin. Countries with social health insurance schemes should adjust accreditation and payment systems to drive coverage of quality TB care. Strong systems for quality assurance and audit are integral to all such approaches to avoid perverse incentives.
- NTPs and their technical partners should improve their understanding of mixed health systems by gathering and using new kinds of data (table 1.3.3). Examples include the number, location, and basic characteristics of all private providers, formal and informal; patterns of care-seeking behavior and its determinants, disaggregated by socioeconomic group; patterns of provider behaviors related to TB, and their determinants; and trends of anti-TB drug sales in private markets.
- NTPs should accelerate the deployment of case-based digital data systems for notifying TB cases and monitoring their care. This benefits all providers, but is especially important for engaging private providers.

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1.5 Tackling drug resistance

Over the next decade, at least 6 million people are projected to develop drug-resistant tuberculosis (DR-TB). At current levels of treatment provision and success, most of these people will die from TB, with many transmitting their DR infections to others before they succumb. By 2050, one-fourth of the predicted 10 million annual deaths attributable to antimicrobial resistance (AMR) globally are expected to be due to DR-TB, which will make it the leading cause of AMR-related death and *Mtb* the most significant airborne pathogen that is drug-resistant.[58] Given these projections, addressing TB drug resistance is essential both for curtailing the global AMR crisis and ending TB. Although providing universal drug resistance testing and scaling up access to high-quality, tailored treatment for DR-TB will require substantial funding and commitment, the consequences of not doing so would be enormous, including massive loss of life and trillions of dollars spent as multidrug-resistant TB (MDR-TB) increases dramatically.[58] Modelling analysis commissioned for this report demonstrates the impact of ensuring universal accesss to DST and second-line therapy in a high DR-TB country, like Moldova. As highlighted in Figure 1.5.1~~3~~, optimizing access to DST and increasing treatment success rates would lead to a 43% reduction in TB mortality and 73% reduction in incidence over the next ~~thirty~~30 years. While these interventions alone would not end the DR-TB epidemic in that setting, with adequate investment in tools, the prospect of definitively addressing the threat of DR-TB within a generation is credible.

Encouragingly, the rapidly evolving field of DR-TB diagnostics and the increasing availability of new and repurposed drugs and regimens for treating patients with multidrug-resistant/rifampicine-resistant (MDR/RR) TB present opportunities to dramatically improve the epidemic response (Table 1.5.1). Emerging data suggest that in high-burden settings, more than 90% of incident MDR/RR-TB disease results from direct transmission of already resistant TB bacteria from one person to another.[59,60] As a result, failure to diagnose and effectively treat a significant proportion of active TB cases is a major driver of the epidemic. Barriers to diagnosis and treatment scale-up vary across countries but include 1) the high cost of providing treatment (although data show such costs can decrease dramatically when more individuals are offered access[61]); 2) perceived complexity of treatment regimens; 3) poor programmatic treatment outcomes; 4) reliance on centralized and specialized treatment; and 5) lack of political will and commitment.[62-65]

Because most DR-TB is caused by direct transmission, early diagnosis and initiation of effective therapy should be the first priority in preventing DR-TB[66].[58] However, reducing the risk of further resistance

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development, particularly to new TB drugs, is also of concern, with data suggesting that when TB drugs are used as "last resort options," resistance may more likely develop.[67] In this regard, strategies for implementing new TB regimens need to take into account the factors that led key first-line drugs to acquire resistance in the past. Robust stewardship mechanisms, especially in the private sector, is crucial in this regard.

Such factors include varying individual pharmacokinetics, comorbidities (particularly those that may affect drug absorption, e.g., HIV), poor drug quality, inadequate prescribing, and selective treatment adherence.[68-72] Weak health systems that offer limited support for patients and their families contribute to many of these factors, emphasizing the importance of strengthening health systems to help respond to the DR-TB epidemic.[73-75] Because TB drug resistance emerges spontaneously and likely will be selected for during treatment,[76] using standard combination regimens in patients with undiagnosed drug resistance likely will contribute to further resistance acquisition, in addition to poor patient outcomes.[77-82]

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1.5.1 Universal access to drug susceptibility testing

Given the clear requirements to find and treat all individuals with DR-TB, and prevent the emergence of further resistance, universal drug sensitivity testing (DST) (to rifampicin as a minimum) is a key recommendation of this Commission. Until relatively recently, diagnosis of DR-TB relied on TB culture, with consequent long delays and need for specialised laboratories.[83] Because DR-TB results from the presence of resistance-conferring mutations in the bacterial genome, newer tests, such as the Xpert MTB/RIF test[84] and line probe assays[85], rely on identifying mutations known to infer drug resistance. These more rapid tests have shortened the time required to receive results from months to hours,[84,86] consequently reducing how long it takes to initiate treatment across a range of settings.[87,88] Newer versions of these and related tests are expected to expand the range of drugs that can be tested and reduce reliance on specialised laboratories.[89-91] A pipeline of candidate point-of-care diagnostics, implemented at the same time as an initial health care visit, have the potential to dramatically improve case detection and reduce losses along complicated diagnostic and care cascades.[92,93]

1.5.2 Improving DR-TB treatment

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The high MDR/RR-TB burden and poor patient outcomes highlight the dire need for more safe, effective, less toxic, shorter, tolerable, and less costly treatment regimens for MDR/RR-TB.[94-98] Encouragingly, two new TB drugs (bedaquiline and delamanid) have received conditional recommendations from WHO for use in MDR/RR-TB treatment.[99-101] These drugs, along with drugs repurposed for TB (including linezolid and clofazimine) and pretomanid (a similar drug to delamanid), are included in a range of new, shorter, all-oral regimens currently being tested in clinical trials~~led~~ for MDR/RR-TB treatment.[102] Results from most of these trials, however, are not expected for several years.[103] In the meantime, these new and repurposed drugs are increasingly being used programmatically. Data from South Africa suggest dramatic improvements in mortality and reductions in treatment failure among more than 3,000 patients treated with bedaquiline to date.[104]

However, currently these new drugs can be used only in patients with additional second-line resistance or at risk of severe adverse events with existing drugs. While such patients undoubtedly benefit, these new drugs also offer the best chance to cure all patients with MDR/RR-TB. Substantially more data exist to support the use of these two and some of the repurposed drugs in DR-TB than we have data to support the use of existing second-line drugs.[105-110] Increasing the use of these new drugs would remove reliance on some more toxic and less effective drugs, including the second-line injectable agents, which are associated with irreversible hearing loss in many people who receive them. It also would help relieve the burden on the health care system to deliver the daily injections.[111] A more individualized approach to DR-TB treatment—one that encompasses access to all available second-line drugs and is guided by more extensive DST through whole genome sequencing—would enable individuals with DR-TB to receive the best chance of cure, while limiting both the unnecessary use of toxic drugs and resistance amplification.[112] Such an approach would need to be supported by implementation research to guide its integration into existing TB programmes and the health system as a whole, in addition to pharmacovigilance systems.[103,113,114]

Given the arduous nature of current TB treatment regimens as well as socioeconomic challenges, many patients withdraw from treatment before completing the full course: globally reported as 15% in the 2014 cohort, and ranging between 1% and 56% in individual studies, with a tendency to increase as more patients are treated in a particular setting.[94,115] These data emphasize the need for more patient- and household-centered approaches. This includes models that flip the current approach on its head by focusing more on the needs of the population affected by DR-TB as opposed to the needs of the health

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systems and practitioners providing them with care (Figure 1.5.0). While the emphasis has been on improving adherence and reducing catastrophic costs, a person-centred model of care also includes ensuring that people with possible DR-TB (and those supporting them) are fully informed about, and included in, therapeutic decisions. ~~At their heart, s~~Such models must tackle active discrimination within the health system as well as in other sectors. Person-centred care also includes providing treatment closer to where patients live and initially seek care, i.e., community-based and decentralised as much as possible.[116] Such models of care should also provide counselling and treatment literacy, along with economic support where needed.

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1.5.3 Preventing resistance acquisition

While diagnosis and prompt treatment are central to tackling the TB epidemic, minimizing the risk of further resistance acquisition, both to existing first- and second-line drugs and new drugs, is also paramount. This includes addressing the drivers of TB drug resistance listed above through programmatic quality improvement (Section 1.1), but also avoiding the use of standardized regimens in the absence of DST wherever possible. Finally, antibiotic stewardship entails ensuring that new drugs are used in tailored, effective multidrug regimens for all patients with DR-TB, not just those with limited therapeutic options. Such use also needs to be supported by expanded TB drug-resistance surveillance (to replace intermittent, expensive DR-TB surveys).

As with drug-susceptible TB, treatment of latent DR-TB may significantly impact the epidemic in the long term. Currently at least two trials are evaluating different prevention regimens for individuals in close contact with MDR/RR-TB patients.[102] In addition, WHO released a conditional recommendation in 2018 supporting the use of individualized preventive treatment for contacts of MDR/RR-TB patients who are at high risk of progressing to disease.[117] Given the high morbidity and mortality associated with DR-TB, preventive treatment of these high-risk contacts, including children and people living with HIV, is a priority.

1.5.4 DR-TB a global health security threat – implications for donor financing

The cost of treatment for MDR/RR-TB, ranges from estimates of US$1,218 in low-income countries to US$83,365 in high-income countries[118]. The high cost has been a significant barrier to scaling up treatment to date. The Stop TB partnership estimated that in 2017, US$2 billion was required to fund DR-TB care; it is expected to increase to US$3.6 billion by 2020.[94] Funding at this level is unlikely to be

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sustainable for many high MDR/RR-TB burden countries; the BRICS countries (Brazil, the Russian Federation, India, China, and South Africa) are notable exceptions. As a result, funding to support DR-TB programme implementation will likely be required from international sources, even in countries with the capacity to fund their own DS-TB programmes. The cross-border threat posed by DR-TB, provides a compelling rationale to justify increased donor financing, even in middle-income countries transitionging out of donor eligibility[119,120]. We discuss the implications of this further in Section 3. .

1.5.5 Recommendations

- Provide universal access to drug susceptibility testing (DST, as a minimum to rifampicin) for all individuals diagnosed with TB and to second-line DST for all individuals diagnosed with RR-TB.
- Treat all individuals with MDR/RR-TB with regimens that include new and repurposed drugs to give the best chance of cure and provide preventive treatment to high-risk contacts of individuals with DR-TB.
- Align the global response to DR-TB with the global Antimicrobial Resistance (AMR) and global health security agendas.

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Tables & Figures

Figure 1.1 Prioritizing policy Interventions

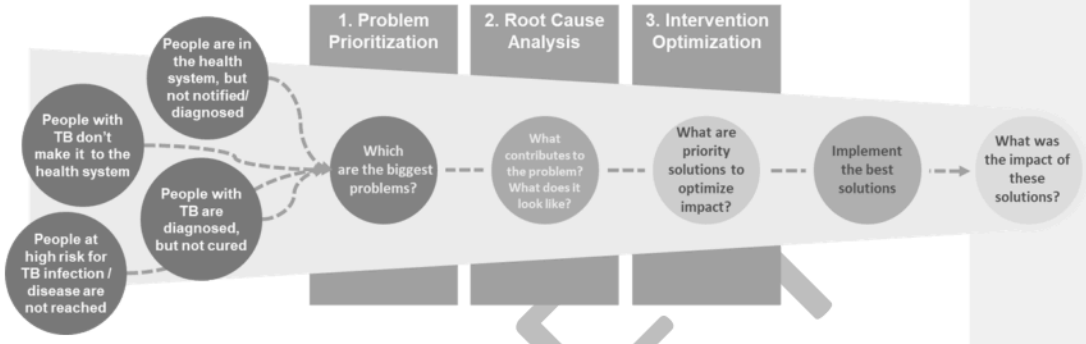

Figure 1.1.2 . Setting patient-centered outcomes using epidemiological, patient pathway, and system data

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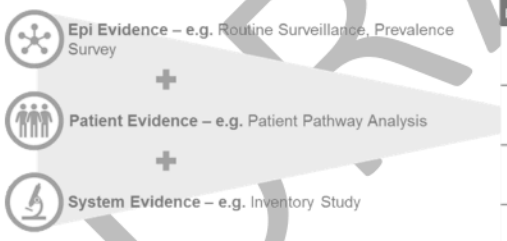

Patient / Epi	Patients don't make it to the health system	Patients in the health system, but not notified/ diagnosed	Patients are notified, but not cured	Total
DS-TB				%
DR-TB				%
TB/HIV				%
Asymp. TB				%
Total	%	%	%	100%

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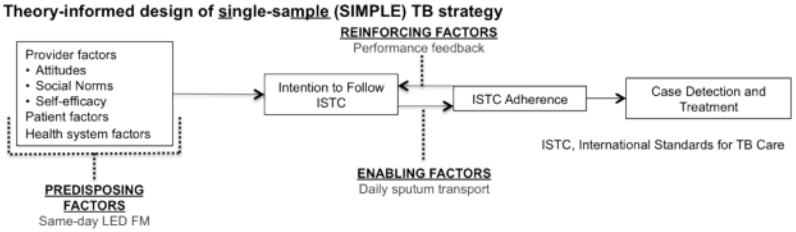
In Ugandan health centers, Davis and colleagues had previously documented major losses during the care cascade, with at most 34% of patients with confirmed TB getting diagnosed and treated (Davis et al. AJRCCM 2011). To address specific barriers leading to drop-out during the cascade of TB care, the project team used a theory-informed approach that accounted for predisposing, reinforcing and enabling factors (graphic) to develop a streamlined strategy called SIMPLE TB for improving TB diagnostic evaluation and treatment initiation. The SIMPLE TB strategy included: 1) examination of two smear results from a single spot sputum specimen using LED fluorescence microscopy, 2) daily transportation of smear-negative sputum samples to Xpert® MTB/RIF testing sites, and 3) performance feedback.

Theory-informed design of single-sample (SIMPLE) TB strategy



Performance feedback alone improved the probability of patients with confirmed TB getting diagnosed and treated to 67% (Chaisson et al. PLoS One 2015). The other two components of the SIMPLE TB strategy were evaluated in a pilot study including 1212 adults who underwent sputum testing for TB (Shete et al. Int J Tuber Lung Dis 2017). As shown in the Table below, the overall probability of patients with confirmed TB getting diagnosed and treated rapidly was 84%, which is much higher than the 34% probability in previous analyses.

1212 adults undergoing TB evaluation	%	Range
Indicator 1: Proportion referred for sputum-based TB testing	99%	95 – 100%
Indicator 2: Proportion completing TB testing (if referred)	99.6%	99 – 100%
Indicator 3: Proportion treated within 14 days (if smear- or Xpert-positive)	85%	81 – 92%
Indicator 4: Cumulative probability of being diagnosed and treated	84%	76 – 92%

Thus, the SIMPLE TB strategy was feasible to implement, improved quality, and led to successful incorporation of Xpert testing and rapid treatment initiation in the majority of patients with confirmed TB in a resource-limited setting.

Case Study 1: How the TB care cascade was improved in Uganda
Source: Shete, et al (2016)

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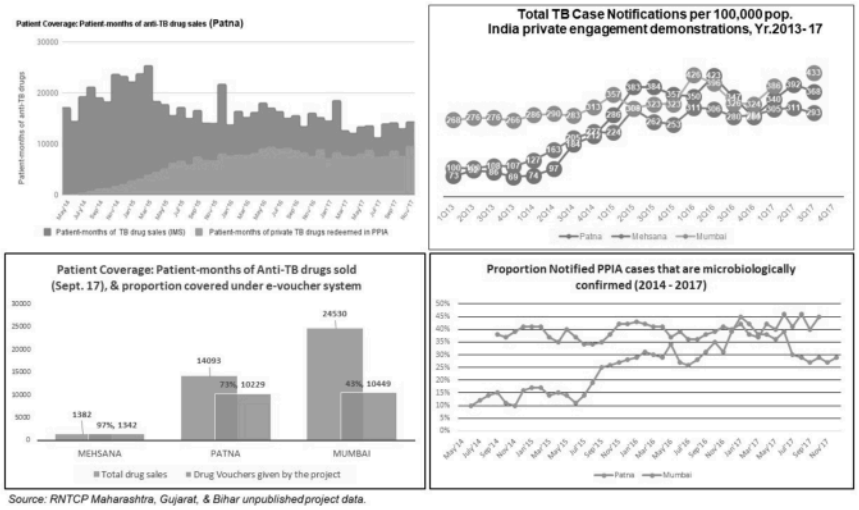
Case Study 2: How quality of TB care was improved in the Indian private sector

Through pilot projects, in Mumbai, Patna and Mehsana in India, the Revised National TB program in India and partners collectively developed and demonstrated an effective solution set for private provider engagement on TB in the Indian delivery environment. Key elements in this model (graphic) include: (1) attracting private providers to report TB with patient retention services, (2) easy digital e-vouchers for free anti-TB drugs and laboratory tests to patients seeking care in private sector, (3) adherence monitoring and support to help patients seeking TB treatment in private sector to complete treatment, and (4) quality of care monitoring and improvement using targeted feedback.

Evaluations and independent research demonstrated 1.5 to 5- fold increases in total reported TB cases, improved quality of care and sputum testing with new diagnostics by providers, and 75% treatment completion (graphic below). This transformation was achieved at cost-per-case ($85-140/case) comparable to that already spent by the public sector program ($110/case in 2015). This effort helped inspire ambition and confidence in the subsequent national planning process, as reflected in the National Strategic Plan for TB Elimination by 2025, which anticipates substantial increases in private sector notifications.



Source: RNTCP Maharashtra, Gujarat, & Bihar unpublished project data.

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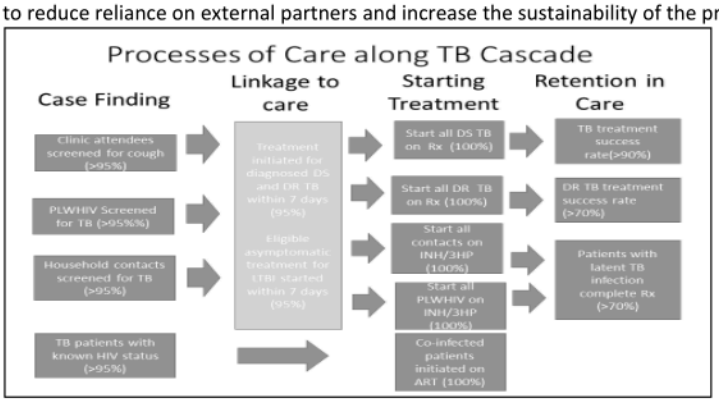
Case Study 3: How South Africa is implementing a QI program

In 2017, South Africa published its new National Strategic Plan for TB, HIV and STIs (2017-2021). As part of this NSP, the South African Department of Health launched a QI program, in collaboration with partners such as GFATM, IHI, BMGF and USAID-funded implementing partners to address the gaps identified in the cascade of TB care (graphic). The goal is to use an established QI methodology to improve quality of TB services to reduce mortality by 50% and incidence by 30% by 2022. The first phase of the project involved training, deep engagement and support to 10 high burden sub districts (266 facilities) with a view to harvesting the most impactful interventions to close gaps in the care cascade. These interventions are then compiled into a change package, and systematically scaled up nationally over a 4 year period using the IHI BTS model, with an initial focus on high burden geographies. The project is intentionally designed to build capacity at the district management level to reduce reliance on external partners and increase the sustainability of the project.



In addition to training of a large number of health workers, facilities were supported in the development of facility plans and mentored during implementation. They established a core set of indicators to track processes along the cascade for use at facility level. They also developed guides for district and facility staff on QI implementation, data management and clinical practices. Good, reliable and regularly collected data is central to the success of such an initiative and much attention is given to designing a simple, but adequate M&E framework for the project. As part of the facility-based QI, barriers for completing the care cascade are identified and interventions are tried out.

High levels of national and district engagement resulted from initial training sessions on change management and QI methods. The implementation phase began with learning sessions in May 2017, and high levels of activity and process testing are underway. Many clinics are showing rapid improvement in TB screening. The SA DOH expects to see an improvement in quality, across the cascade of care in 2018 and plans to finalize the change package for scale up commencing 2019.

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Table 1.1.3 Suggested indicators to be included in a QI program at the health facility level

Step in the cascade of care	Indicator
Symptom screening	% of clients in the clinic that are screened for cough >2 weeks and other TB symptoms
TB testing	% of TB symptomatics that are referred for TB testing
Diagnosis	% of TB symptomatics that complete TB testing algorithm and get diagnosed
DST	% of diagnosed TB patients who get rapid test for rifampicin resistance
Assessment of comorbid conditions	% of diagnosed TB patients who get tested for HIV
Initiation of anti-TB treatment	% of diagnosed TB patients who get initiated on anti-TB treatment within 7 days
Correctness of TB treatment	% of TB patients who get the correct/appropriate anti-TB drug regimen
Completion of anti-TB treatment (retention in care)	% of patients initiated on anti-TB treatment that complete therapy
Household contact screening	% of household contacts screened for TB
Assessment of risk-groups for preventive therapy	% of PLWHIV screened for TB infection and initiated on preventive therapy

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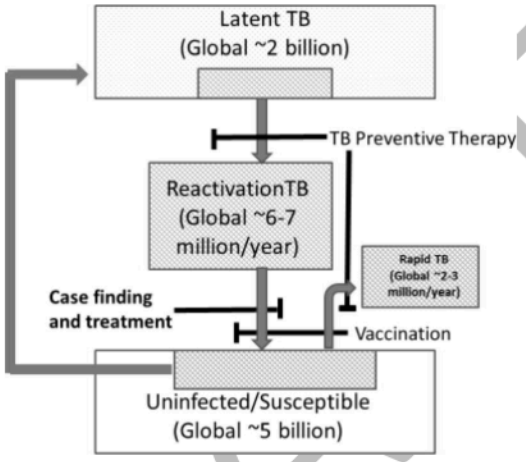
Figure 1.3.1. Global dynamics of TB infection and disease



Source: Rangaka et al (2015)

Notes: Diagnosis and treatment of active TB cases reduces onward transmission of infection and over a period of decades can slowly eliminate the pool of latent infections, but for many years new cases will continue to arise from the latent infection pool. Treatment of latent infection in these individuals and in those who are newly infected due to contact with a case can substantially reduce incidence rates.

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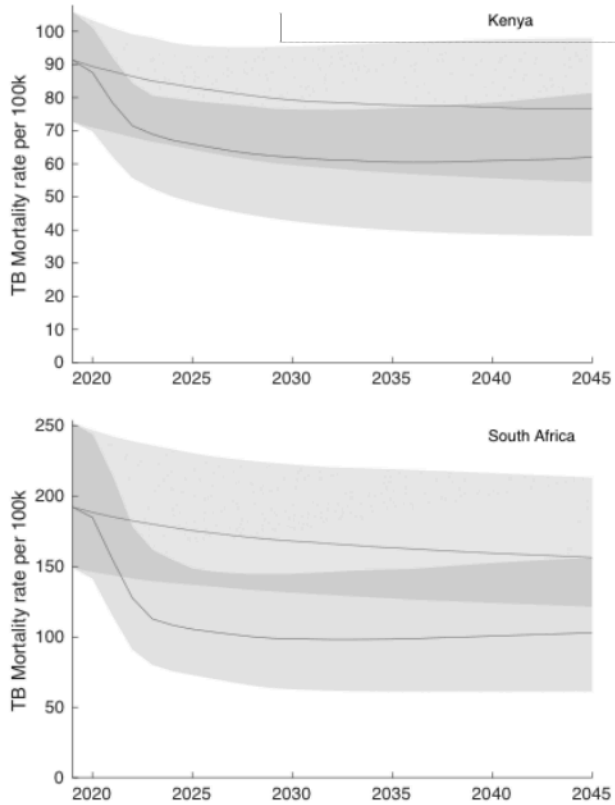

Figure 1.3.1. The potential impact of improved collaboration between HIV and TB programmes. Shown are mortality projections in two high-HIV burden scenarios: settings consistent with Kenya and South Africa, where the proportion of TB cases that are HIV coinfected are respectively 16% and 60%. In both settings the intervention involves an increase in ART coverage from current levels to 90%, and an increase in isoniazid preventive therapy amongst those in HIV care from current levels to 90%. The overall impact of these is to reduce mortality by 33% (95% CrI 18 - 49) in a setting like Kenya, and by 46% (95% CrI 30 – 62) in a setting like South Africa. In the example of Kenya, these measures will involve a mean incremental cost of USD 66m (95% CRI 20m – 113m) per annum between 2018 and 2045 to the TB programme, excluding the costs of HIV activities, and of managing drug-resistance TB. Inclusion of the latter is likely to reduce the incremental cost, owing to the reduced burden of MDR-TB. See (ref – parallel modelling paper) for further details on the modelling approach used.

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Table 1.3.2. Barriers and obstacles to implementing TB preventive therapy and potential solutions.

	Challenges	Strategies
Clinical	Excluding active TB	Use of clinical algorithms, x-rays
	Need for TST of IGRA	POC test, clinical screening
	Poor adherence	Short course regimens
	Drug toxicity	Safer regimens
	Perceived risk of 'creating resistance'	No evidence of a problem!
	Reinfection (esp for HIV+)	Lifelong preventive therapy
Policy and Advocacy	Lack of priority for prevention	Realignment of TB control programs to incorporate prevention, with performance assessments
	Inadequate investment in research and training	Increased funding for basic and targeted research
	Absence of advocacy from groups most at risk	Education and empowerment of at-risk groups, including people with HIV and families
Health System	Absence of consistent guidelines	Harmonize global guidelines
	Inadequate number of trained clinicians	Enhanced training, task-shifting
	Stock outs of diagnostics and drugs	Strengthened supply chain, Global Drug Facility
	Poor surveillance and reporting	Better health information systems
	Inadequate funding	Expansion of vertical programs to include TB

Source: Rangaka et al (2015)

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HHS-NIAID-20-0593-A-000025

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HHS-NIAID-20-0593-A-000026

Table 1.3.2. Populations that can benefit from TB Preventive Therapy and responsibility for delivery within the health care system

Population	Risk for progression	Global numbers	Responsibility for delivery	Reference
HIV infection	2-10% per year	~30 million	National AIDS Programs	UNAIDS
Household contacts <5	5-40% over 2 years	~5.5 million per year	Primary Health Care, TB Programs, MCH Programs	Dodd et al (2014)
Household contacts >5	Up to 10% over 2 years	~20 million per year	Primary Health Care, National TB Programs	Fox et al (2018); Morrison et al (2008)
Health care workers in high-burden settings	1-3% per year, variable	>Millions	National and Local Health Systems	
Prisoners in high-burden settings	1-10% per year	>Millions	Correctional authorities	

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HHS-NIAID-20-0593-A-000027

Table 1.4.1: Private provider engagement as a disruptive innovation for National TB Control Programs

Feature	Current operating model	Disruptive operating model
Implicit mission and role	Care for TB patients who seek care at public facilities; act as service provider	Care for all TB patients regardless of where they seek care; act as service enabler
Assumed channel to deliver care	Public sector providers and facilities responsive to NTP direction	All providers and facilities, prioritized according to patient preferences
Approach to private providers and markets	Compete with and mistrust of private providers; deny or combat market forces	Collaborate and partner with private providers as indicated by their role in health market system; acknowledge and harness market forces
Mental map for engagement	Preference for standardization; top-down, policy-driven innovation; linear systems	Comfort with ambiguity and adaptation; innovation as emergent evolutionary process; complex adaptive systems
Tools for engagement	Mainly internal: forms, standardized procedures, instructions	Mainly external: incentives, enablers, contracts
Core skills and competencies	Line management, supervision, logistics Staff and leadership medical	Negotiation, influence, purchasing Staff and leadership with balanced expertise including social science, health systems, health finance
Data for decision-making	Overwhelmingly medical data: Service statistics on case-notification and treatment outcomes. Weak/inconsistent data on source of notification and referral; no data on resource allocation by type of provider	Balance of medical and socio-economic data, including: Numbers, characteristics, location and TB services of all kinds of providers; patient care-seeking pathways and preferences; provider behaviors and motivations; private drug sales

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		volumes and trends; resource allocation by type of provider
Funding for private provider engagement	Ad hoc, short-term, small-scale, external; optional extra first to be cut	Integrated into core budgets; long-term; large-scale; domestic and external
Financing modalities	Input-based, supply-side, historical	Performance-based, often demand-side

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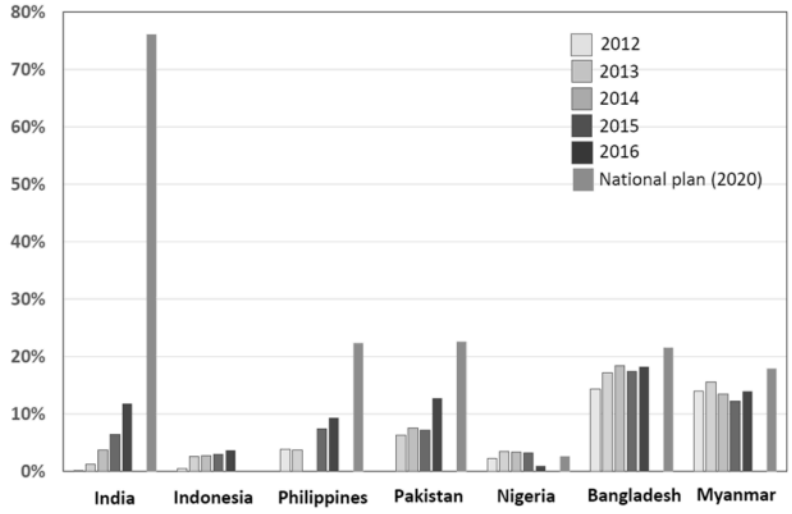
HHS-NIAID-20-0593-A-000030

Figure 1.4.1. The percentage of privately notified TB patients among estimated number of TB patients from 2012-2016 in 7 high TB burden countries with dominant private healthcare systems



Note: When available in the National Strategic Plan for TB, this percentage is presented as the 2020 target.

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HHS-NIAID-20-0593-A-000031

Panel 1.4.1 Progress in scaling up private provider engagement in India

Foundation for change (2012)

- Making TB notification mandatory
- Development of a case-based electronic TB case notification system (*Nikshay*)
- Issuance of decrees banning serological tests for TB
- Decision to align NTP regimens with those commonly used in the private sector by adopting daily fixed-dose combination (FDC) TB drugs
- Private notifications accounted for 1-2% of estimated incident cases in 2012

Demonstration of scalable interventions (2014-16)

- Starting in 2014, programs in Patna, Mumbai, and Meshana began to demonstrate a scalable model for private provider engagement
- Three key interventions
    - Attract private provider notifications using call centers and new ICT system
    - Support the private providers to retain their patients using eVouchers for free drugs and diagnostics and monitoring patients for adherence
    - In urban areas with high density of private providers, fund intermediary agencies to engage providers, collect notifications, and support patients during treatment; in rural areas with lower density of private providers, public program performs functions of intermediary agencies
- Demonstration programs reported 1.5-5 times increase in total TB notification

Political commitment and funding for scale-up (2017-18)

- Increased political commitment to address TB by Prime Minister Modi; regular review of progress in TB control by the Prime Minister Office
- Minister of Health meeting monthly with states on progress in TB, including private provider notifications
- National Strategic Plan for TB (2017-2020) calls for a six-fold increase in private notifications to two million cases per year by 2020 (~75% of estimated incidence)
- Government allocates a three-fold budget increase for TB ($2 billion for 2017-19)

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of the Freedom of Information and Privacy Act

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Fri, 20 Apr 2018 22:06:46 +0000
To: Conrad, Patricia (NIH/NIAID) [E]
Subject: RE: VOA Plugged In Interview Request for Dr. Anthony Fauci

Thanks. I thought so, but the e-mail at the bottom (see second yellow highlight) confused me.

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Friday, April 20, 2018 6:05 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: Re: VOA Plugged In Interview Request for Dr. Anthony Fauci

They are coming to your office we never have u go downtown.

Sent from my iPhone

On Apr 20, 2018, at 6:04 PM, Fauci, Anthony (NIH/NIAID) [E] (b)(6) wrote:

Let us discuss. Are they coming here to do the interview? I am not that happy about going down to Independence Avenue. I forgot whether I agreed to go down to Independence Avenue

---

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Friday, April 20, 2018 3:09 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: FW: VOA Plugged In Interview Request for Dr. Anthony Fauci

Fyi see highlight.

Patricia L. Conrad
Special Assistant to the Director

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From: Jean Michel Mathurin <jmathuri@VOANews.com>
Sent: Friday, April 20, 2018 3:06 PM
To: Routh, Jennifer (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Joan DeLuca (b)(6) Abdul Aziz Khan (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Subject: Re: VOA Plugged In Interview Request for Dr. Anthony Fauci

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Hi Jennifer,

Thank you for getting back to me. Greta Van Sustern will be conducting the interview. It will remain largely around the topics previously discussed. I think it will 2 vehicles, sometimes Greta drives, I am checking on that now. The other will be the (b)(6) (b)(6) My cell number is (b)(6)

Mino Dargakis
Hakim Shammo
Thomas Bagnall
Jean Michel Mathurin

Greta Van Susteren

Jean Michel Mathurin
4439-C
Director/Producer
Off (b)(6)

From: Jean Michel Mathurin [mailto:jmathuri@VOANews.com]
Sent: Monday, April 2, 2018 4:22 PM
To: Routh, Jennifer (NIH/NIAID) [E] (b)(6)
Cc: Joan DeLuca (b)(6) Abdul Aziz Khan (b)(6)
Subject: Re: VOA Plugged In Interview Request for Dr. Anthony Fauci

Hi Jennifer,

Thank you for responding to our request. The 20-minute interview would be recorded in-person in our studio at 330 Independence Ave. Topics we will discuss are; pandemic preparedness, vaccine development, Cholera, and Progress against HIV/AIDS. We have several days available, depending on Dr. Fauci's schedule:

- Thursday 4/5/18

- Monday 4/9/18

- Tuesday 4/10/18

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Please let me know what day works best so we can arrange a specific time with our studio crews. Thank you, kindly. We look forward to hearing from you soon.

Best,

Jean Michel Mathurin
4439-C
Director/Producer
Off 202-382-7069

From: Routh, Jennifer (NIH/NIAID) [E] (b)(6)
Sent: Monday, April 2, 2018 2:09:26 PM
To: Jean Michel Mathurin
Subject: RE: VOA Plugged In Interview Request for Dr. Anthony Fauci

Hello,

I wanted to get a few more details regarding this request. Which diseases and topics would the interview cover specifically? Also, what time and day is the show recorded, and approximately how long would an interview take? Also, are all shows recorded in-person at the Independence Ave. studio?

Thanks,
Jen

Jennifer Routh [E]
News and Science Writing Branch
Office of Communications and Government Relations
National Institute of Allergy and Infectious Diseases (NIAID)
NIH/HHS
31 Center Drive Room 7A17C
Bethesda, MD 20892
Direct: (b)(6)
(b)(6)

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From: Jean Michel Mathurin [mailto:jmathuri@VOANews.com]
Sent: Monday, April 02, 2018 1:34 PM
To: NIAID NEWS (NIH/NIAID) (b)(6)
Subject: VOA Plugged In Interview Request for Dr. Anthony Fauci

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Greetings,

I am writing on behalf of Greta Van Susteren's new television show "Plugged in with Greta" produced by Voice of America. It is an internationally distributed broadcast to a worldwide audience. Previous guests on Plugged in include; Vice President Mike Pence, U.N. Ambassador Nikki Haley and former National Security Advisor General H.R. McMaster. For an upcoming broadcast, Greta would love to have Director, Dr. Anthony S. Fauci on the show to discuss different issues related to infectious diseases. I am hoping you can help me reach the proper contacts to discuss the details of this segment on "Plugged in". Attached, you will find a PDF flyer for the show. I would love an opportunity to discuss how we can make this interview a reality. I look forward to hearing from you soon.

Respectfully,

Jean Michel Mathurin

4439-C
Director/Producer
Plugged in with Greta
VOA 330 Independence Ave. Washington, DC 20237
Off (b)(6)

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Fri, 20 Apr 2018 20:30:04 +0000
To: Borio, Luciana L. EOP/NSC
Subject: RE: flu

Thanks, Lu. Hopefully, the fact that we both brought it up will have some effect.
Best regards,
Tony

From: (b)(6)
Sent: Friday, April 20, 2018 4:12 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Lane, Cliff (NIH/NIAID) [E] (b)(6)
Subject: flu

Thank you for raising the sample sharing / H7N9 issue in your discussion with Tedros. We met with him today, raised it as well, and he mentioned that you also pressed.

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From: Arms, Erin (NIH/OD) [E]
Sent: Mon, 16 Apr 2018 19:17:49 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Accepted: Biodefense hearing pre-brief (2:15pm)

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Mon, 16 Apr 2018 15:09:31 +0000
To: Fauci, Anthony (NIH/NIAID) [E];Bryant, Paula (NIH/NIAID) [E];Johnson, Martin S. (NIH/NIAID) [E];Haskins, Melinda (NIH/NIAID) [E];Selgrade, Sara (NIH/NIAID) [E];Harper, Jill (NIH/NIAID) [E];Erbelding, Emily (NIH/NIAID) [E];Cassatt, David (NIH/NIAID) [E]
Cc: Glowinski, Irene (NIH/NIAID) [E];Rotrosen, Daniel (NIH/NIAID) [E];Arms, Erin (NIH/OD) [E]
Subject: Biodefense hearing pre-brief (2:15pm)
Attachments: FW: Requesting assistance: Biodefense hearing pre-brief next week

Toll Free Number: (b)(6)

Participant Passcode: (b)(6)

Leader Passcode: (b)(6) – ASF only

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Fri, 13 Apr 2018 20:57:17 +0000
To: Vance, Meaghan (NIH/NIAID) [C]
Subject: FW: Requesting assistance: Biodefense hearing pre-brief next week

Lets look at the calendar Monday to see when to schedule this.thx

Patricia L. Conrad
Special Assistant to the Director

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From: Selgrade, Sara (NIH/NIAID) [E]
Sent: Friday, April 13, 2018 4:54 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: NIAID OCGR Leg (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6) Billet, Courtney (NIH/NIAID) [E] (b)(6)
Subject: Requesting assistance: Biodefense hearing pre-brief next week

Hi Patty,

Thanks very much for your help in arranging this call for next week. Details of the pre-brief as discussed this afternoon are below. Please let us know if you need anything else.

Thanks,
Sara

Biodefense Hearing Pre-brief
45-minute phone call

Invited: Paula Bryant, David Cassatt, Jill Harper, Martin Johnson, Melinda Haskins, Sara Selgrade
Copied on Invitation: Emily Erbelding, Irene Glowinski, Daniel Rotrosen

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From: Harper, Jill (NIH/NIAID) [E]
Sent: Mon, 16 Apr 2018 15:47:13 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Harper, Jill (NIH/NIAID) [E]
Subject: Accepted: Biodefense hearing pre-brief (2:15pm)

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From: Johnson, Martin S. (NIH/NIAID) [E]
Sent: Mon, 16 Apr 2018 15:40:06 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Accepted: Biodefense hearing pre-brief (2:15pm)

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From: Haskins, Melinda (NIH/NIAID) [E]
Sent: Mon, 16 Apr 2018 15:36:49 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Accepted: Biodefense hearing pre-brief (2:15pm)

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From: Bryant, Paula (NIH/NIAID) [E]
Sent: Mon, 16 Apr 2018 15:15:50 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Accepted: Biodefense hearing pre-brief (2:15pm)

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From: Cassatt, David (NIH/NIAID) [E]
Sent: Mon, 16 Apr 2018 15:11:08 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Accepted: Biodefense hearing pre-brief (2:15pm)

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From: Selgrade, Sara (NIH/NIAID) [E]
Sent: Mon, 16 Apr 2018 15:10:00 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Accepted: Biodefense hearing pre-brief (2:15pm)

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From: Chris Collins
Sent: Thu, 12 Apr 2018 15:35:04 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: At the tipping point for U.S. leadership on AIDS, TB & malaria

Dr. Fauci, I look forward to seeing you tonight at CSIS.

I wanted to let you know that today *Friends of the Global Fight* released a new report -- **At the Tipping Point: U.S. Leadership to End AIDS, Tuberculosis and Malaria.** It is available here.

It shows (1) the considerable progress U.S. leadership has won on the 3 epidemics and (2) that a stepped-up global effort is needed to end these epidemics for good. Using a concise and visual approach, it looks at the *status of the three epidemic responses*, *the U.S. role*, the *big challenges*, and the *opportunities for increased impact*.

For all the considerable progress, *At the Tipping Point* argues that decision makers have a **critical choice of options** to make: to mobilize partners and accelerate toward the end of epidemics, or stall and risk allowing disease resurgence and drug resistance to overpower success to date.

My companion oped in *The Hill* is below and here.

Thanks,

Chris

American leadership against deadly epidemics reaches a decision point

By Chris Collins

TheHill.com April 12, 2018

What is the end goal of America's considerable investment in tackling the deadliest infectious diseases of our time? Americans should be proud that U.S. leadership against AIDS, tuberculosis (TB) and malaria has saved tens of millions of lives and helped dramatically to reduce their devastation. But what should be the long-term objective of these investments?

Whether you are looking from the perspective of lives saved, U.S. health security, global stability, economic growth, or American trade interests, the answer is clear: We need to focus on ending these three epidemics for good.

The epidemics of AIDS, TB and malaria are each at a tipping point. Thanks to global efforts over the past 15 years, disease incidence and mortality have been driven

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downward. Twenty-one million people are now receiving lifesaving treatment for HIV. Between 2014 and 2016 alone, 582 million insecticide-treated nets were distributed globally to protect children and their families from malaria.

An estimated 53 million lives have been saved through TB diagnosis and treatment so far in the 21st century. In the process of scaling up targeted approaches to these diseases, health systems have been strengthened and are now more ready to identify and respond to the next Ebola or Zika. And many of the countries most heavily affected have increased investment in the health of their own people.

Still, there are serious risks of tipping backward. The number of malaria cases actually increased over the past year, recalling the numerous times in history when malaria resurged after policymakers lost interest. Drug resistance is on the rise for first-line medications used to treat each of the three diseases. And with growing global alarm about the threat of antimicrobial drug resistance, it's important to remember that TB is both the world's deadliest infectious disease and accounts for more than 1 in 4 antimicrobial resistance fatalities each year.

All this means that American policy is at a tipping point, too. Today the world is underinvesting in efforts to end the biggest epidemics; an expanded effort is needed to get on track. We face a choice: to mobilize partners to accelerate toward the end of epidemics, or stall and permit resurgence and drug resistance to overpower our success. Until we intensify the global response, these diseases will continue to claim millions of lives, drain billions of dollars from economies, and put our security at risk.

Moreover, protecting our health security should be a priority. Rapid spread of an infectious disease epidemic is among the most likely scenarios to result in the unanticipated deaths of tens of millions of people in under a year. We need to end the infectious diseases that are killing millions of people today and, in doing so, be better prepared for the diseases of tomorrow.

Other countries can and should do more, but American leadership is essential. The U.S. provides unmatched expertise, sizeable resources and — crucially — strategic resolve to tackle the challenge. Our bilateral global health programs, the President's Emergency Plan for AIDS Relief, the President's Malaria Initiative, and the U.S. Agency for International Development TB program have produced impressive gains. Our investment in the Global Fund to Fight AIDS, Tuberculosis and Malaria leverages increased investment from other donors, the private sector, and implementing countries themselves.

At the Tipping Point, a report released today, details U.S. efforts against AIDS, TB and malaria, and calls for a stepped-up, global effort to end the three epidemics. This would include smart new investments by other countries and the private and philanthropic sectors, matched by modest new resources from our own country, where global health funding has been essentially flat for nearly nine years.

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We should not fool ourselves that ending these epidemics will be easy, but it is possible by scaling up what works and investing in the scientific breakthroughs of the future. And if we do not seize this historic opportunity, achievements from U.S. investments to date could be squandered.

"All infectious diseases will revert and come back," warns Dr. Anthony Fauci, Director of the National Institute of Allergy and Infectious Diseases. "We have seen through multiple experiences over the years; we cannot stop until it's over."

U.S. leadership has catalyzed impressive strides in combatting infectious diseases. Now it is time to focus on ridding the world of the AIDS, TB and malaria epidemics for good, and realize the vast human, economic and security gains this accomplishment will bring. Great nations do great things. Ending these epidemics can be a legacy for the United States. Now is the time to rededicate ourselves to global health leadership, and challenge the rest of the world to join us in a push to the finish line.

Chris Collins is the president of Friends of the Global Fight, which is a U.S. advocacy organization that advocates for the Global Fund to Fight AIDS, Tuberculosis and Malaria.

Chris Collins
President
Friends of the Global Fight Against AIDS, Tuberculosis and Malaria
(b)(6)
T: (b)(6) C: (b)(6)
Web | Twitter

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Wed, 11 Apr 2018 01:23:32 +0000
To: (b)(6)
Subject: Sorry

(b)(6)

(b)(6)

Warm regards,
Tony

Anthony S. Fauci, MD
Director
National Institute of Allergy and Infectious Diseases
Building 31, Room 7A-03
31 Center Drive, MSC 2520
National Institutes of Health
Bethesda, MD 20892-2520
Phone: (b)(6)
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From: Hall, Lee (NIH/NIAID) [E]
Sent: Tue, 10 Apr 2018 17:09:15 +0000
To: NIAID PIPB;NIAID BUGS;Erbelding, Emily (NIH/NIAID) [E];Folkers, Greg (NIH/NIAID) [E];Marston, Hilary (NIH/NIAID) [E];Eisinger, Robert (NIH/NIAID) [E];Meegan, James (NIH/NIAID) [E];Handley, Gray (NIH/NIAID) [E];Billet, Courtney (NIH/NIAID) [E];Fauci, Anthony (NIH/NIAID) [E]
Subject: FW: US Global Malaria Coordinator - Dr. Kenneth Staley

FYI – Kenneth Staley is the replacement for Tim Ziemer, who was the previous US Global Malaria Coordinator for the President's Malaria Initiative.
Lee

From: Martin Alilio (b)(6)
Sent: Tuesday, April 10, 2018 10:35 AM
To: (b)(6) (b)(6) Kilmarx, Peter (NIH/FIC) [E] (b)(6) Hall, Lee (NIH/NIAID) [E] (b)(6) (b)(6) (b)(6) (b)(6) (b)(6) (b)(6) (b)(6) (b)(6) Udhayakumar, Venkatachalam (Kumar) (CDC/CGH/DPDM) (b)(6) FMVCC Information CR ????? hold for more info (b)(6) (b)(6) (b)(6) (b)(6) (b)(6) (b)(6) Rao, Malla (NIH/NIAID) [E] (b)(6) (b)(6) (b)(6) (b)(6)
Cc: Gimnig, John E. (CDC/CGH/DPDM) (b)(6) Julie Wallace (GH/HIDN/ID) (b)(6) Lawrence Barat (b)(6) McElroy, Peter (CDC/CGH/DPDM) (b)(6)
Subject: US Global Malaria Coordinator - Dr. Kenneth Staley

Dear All,

Sharing the news below!

Thanks,

Martin Alilio, PhD
Operational Research Coordinator - President's Malaria Initiative
USAID Bureau of Global Health
Office of Infectious Diseases 10086A
2100 Crystal Drive | Arlington, VA 22202
Office: (b)(6)
Mobile: (b)(6)
(b)(6)

---------- Forwarded message ----------
From: Irene Koek (b)(6)

AMERICAN OVERSIGHT

Date: Tue, Apr 10, 2018 at 8:42 AM
Subject: US Global Malaria Coordinator - Dr. Kenneth Staley
To: "PMI Core Mail List (USAID)" (b)(6) Field Mailing List_PMI (b)(6) (b)(6)
Cc: Rebecca Martin (b)(6) "Parise, Monica E. (CDC/CGH/DPDM)" (b)(6) Alma Golden (b)(6) Kerry Pelzman (b)(6) David Stanton (b)(6)

Dear PMI Team,

It is my distinct honor and pleasure to introduce and welcome Dr. Kenneth Staley as the new U.S. Global Malaria Coordinator with the President's Malaria Initiative (PMI). Dr. Staley's appointment by the White House was announced April 9.

Dr. Staley received his medical degree from the Carver College of Medicine at the University of Iowa. He also earned a Master of Public Administration degree from John F. Kennedy School of Government at Harvard University, and he is an honors graduate of Washington University in Saint Louis.

In the George W. Bush administration, Dr. Staley served in the State Department as Acting Deputy Assistant Secretary of State for Counterproliferation, and as Director for Biodefense Policy at the White House Homeland Security Council. More recently, Dr. Staley has worked on product development and innovation as a Director for Medtronic, and for the last few years he was with McKinsey & Company, where he led teams working on public health and crisis response, including Ebola and Middle Eastern Respiratory Syndrome (MERS).

We anticipate learning more soon on Dr. Staley's start date with PMI and will share this information with you once these details are known. It has been my pleasure and privilege to serve you in the capacity as Acting US Global Malaria Coordinator and I look forward to supporting the PMI Atlanta and Washington leadership team to support a smooth onboarding of Dr. Staley to his exciting new role.

Thank you for your continued contributions to PMI's noble efforts!

Irene

AMERICAN OVERSIGHT

--

Irene Koek
Senior Deputy Assistant Administrator
Bureau for Global Health
USAID
phone: (b)(6)
email: (b)(6)

AMERICAN OVERSIGHT

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Tue, 10 Apr 2018 14:20:16 +0000
To: Folkers, Greg (NIH/NIAID) [E];Fauci, Anthony (NIH/NIAID) [E]
Subject: RE: Per Bloomberg reporter, Tom Bossert to resign

WOW!!!!!

Patricia L. Conrad
Special Assistant to the Director

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From: Folkers, Greg (NIH/NIAID) [E]
Sent: Tuesday, April 10, 2018 10:20 AM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: FW: Per Bloomberg reporter, Tom Bossert to resign

From: Crawford, Chase (NIH/NIAID) [E]
Sent: Tuesday, April 10, 2018 10:14 AM
To: Folkers, Greg (NIH/NIAID) [E] (b)(6) Billet, Courtney (NIH/NIAID) [E] (b)(6)
Cc: NIAID OCGR Leg (b)(6)
Subject: Per Bloomberg reporter, Tom Bossert to resign

AMERICAN OVERSIGHT

From: Folkers, Greg (NIH/NIAID) [E]
Sent: Tue, 10 Apr 2018 14:19:39 +0000
To: Fauci, Anthony (NIH/NIAID) [E];Conrad, Patricia (NIH/NIAID) [E]
Subject: FW: Per Bloomberg reporter, Tom Bossert to resign

From: Crawford, Chase (NIH/NIAID) [E]
Sent: Tuesday, April 10, 2018 10:14 AM
To: Folkers, Greg (NIH/NIAID) [E] (b)(6) Billet, Courtney (NIH/NIAID) [E] (b)(6)
Cc: NIAID OCGR Leg (b)(6)
Subject: Per Bloomberg reporter, Tom Bossert to resign

AMERICAN OVERSIGHT

From: Billet, Courtney (NIH/NIAID) [E]
Sent: Tue, 10 Apr 2018 14:16:30 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: FW: Per Bloomberg reporter, Tom Bossert to resign

From: Crawford, Chase (NIH/NIAID) [E]
Sent: Tuesday, April 10, 2018 10:14 AM
To: Folkers, Greg (NIH/NIAID) [E] (b)(6) Billet, Courtney (NIH/NIAID) [E] (b)(6)
Cc: NIAID OCGR Leg (b)(6)
Subject: Per Bloomberg reporter, Tom Bossert to resign

AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Wed, 16 May 2018 19:33:41 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Act for NIH
Attachments: RE: Act for NIH event - may 23

385 Russell Senate Office Building

AMERICAN OVERSIGHT

From: Selgrade, Sara (NIH/NIAID) [E]
Sent: Wed, 16 May 2018 19:24:57 +0000
To: Conrad, Patricia (NIH/NIAID) [E]
Subject: RE: Act for NIH event - may 23
Attachments: Fauci Flu Briefing Invitation.docx

Thanks for checking in, Patty. The event will be from 12-1pm – I defer to you on how long you would hold on the calendar for travel, etc.

It will be in (b)(6) I am attaching the event flyer if helpful for your purposes.

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Wednesday, May 16, 2018 3:14 PM
To: Selgrade, Sara (NIH/NIAID) [E] (b)(6)
Subject: Act for NIH event - may 23

Do you have an agenda or timeline for this event? also need room and building location. we are holding 11 am – 2 pm. is it that long?

thx

Patricia L. Conrad
Public Health Analyst and
Special Assistant to the Director
National Institute of Allergy and Infectious Diseases
The National Institutes of Health
31 Center Drive, MSC 2520 - Room 7A03
Bethesda, Maryland 20892
(b)(6)
301-496-4409 fax

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OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Wed, 16 May 2018 18:38:57 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Brief interview with Milken schl of Publ Health External Affairs
Attachments: Re: on behalf of Dr. Anthony Fauci, RE: on behalf of Dr. Anthony Fauci, Re: Dr. Fauci interview at GW SPH on 6/5

Also- Our External Relations team is wondering if Dr. Fauci might spare 15 minutes while he is here for a brief interview. They are putting together a video series highlighting guests to Milken Institute SPH, and would be honored if Dr. Fauci can participate. They are happy to provide the questions beforehand---They said it will be a 'mix of both questions about his research/expertise and his views on the general state of public health (greatest issues and achievements in the space).' Please let me know. I can put you directly in touch with our External Relations director if you'd like.

Contact Matthew golden cell (b)(6)

AMERICAN OVERSIGHT

From: Petersen-Jobsis, Halley
Sent: Wed, 16 May 2018 13:51:07 -0400
To: Conrad, Patricia (NIH/NIAID) [E]
Cc: Paules, Catharine (NIH/NIAID) [E]
Subject: Re: on behalf of Dr. Anthony Fauci

Wow. Sorry about that. Not sure how our comm team missed it! I will update it now.

H

On Wed, May 16, 2018 at 1:45 PM, Conrad, Patricia (NIH/NIAID) [E]
(b)(6) wrote:

I will let you know about the interview but the flyer has the wrong title.

Below is the title for Dr. Fauci's talk.

"Arboviruses in the Americas: Emerging and Re-emerging Threats."

Patricia L. Conrad

Special Assistant to the Director

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From: Petersen-Jobsis, Halley (b)(6)
Sent: Wednesday, May 16, 2018 1:37 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)

Subject: Re: on behalf of Dr. Anthony Fauci

Patty-

Thanks for note. Below is the email invitation that we'll be sending out. Does it look okay to you?

Also- Our External Relations team is wondering if Dr. Fauci might spare 15 minutes while he is here for a brief interview. They are putting together a video series highlighting guests to Milken Institute SPH, and would be honored if Dr. Fauci can participate. They are happy to provide the questions beforehand---They said it will be a 'mix of both questions about his research/expertise and his views on the general state of public health (greatest issues and achievements in the space).' Please let me know. I can put you directly in touch with our External Relations director if you'd like.

Halley

Michael and Lori Milken Dean Lynn R. Goldman

and

Professor of Global Health Jon Andrus

The George Washington University Milken Institute School of Public Health

AMERICAN OVERSIGHT

Cordially invite you to a special event

Prevention and Control of Emerging Arbovirus Infections in Latin America and the Caribbean

An internationally recognized panel of Arbovirus experts will discuss status, progress and challenges around the management, diagnostics, surveillance, and prevention and control strategies of emerging Arbovirus infections, to inform policy decisions in Latin America and the Caribbean.

Keynote presentation by **Dr. Anthony Fauci**

Global Arbovirus Disease Burden: Challenges for Prevention and Control

Plenary sessions include:

- Arbovirus disease burden, prevention and control strategies in Latin America and the Caribbean
- Overview of vaccine research and development: Dengue, Yellow Fever, Zika, and Chikungunya
- Arbovirus field surveillance updates from the Caribbean, Brazil, Mexico, and Guatemala
- Considerations in vaccine delivery strategies

June 5, 2018

8:30 am - 5:30 pm | Auditorium

Milken Institute School of Public Health

950 New Hampshire Ave, NW

Washington, DC

AMERICAN OVERSIGHT

RSVP

On Sat, May 12, 2018 at 7:03 AM, Conrad, Patricia (NIH/NIAID) [E]
(b)(6) wrote:

Fine to use his name and photo then

Sent from my iPhone

On May 11, 2018, at 1:48 PM, Petersen-Jobsis, Halley (b)(6) wrote:

No, not a fundraiser. It's a free symposium/forum that we are hoping to open to the public.

I will send over a proof of our promo next week for you to approve.

Thanks and have a great weekend!

H

On Fri, May 11, 2018 at 9:04 AM, Conrad, Patricia (NIH/NIAID) [E]
(b)(6) wrote:

It is not a fundraiser right? IF not then yes – it should be fine. can we see the flyer for final approval?

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Patricia L. Conrad

Special Assistant to the Director

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From: Petersen-Jobsis, Halley (b)(6)
Sent: Thursday, May 10, 2018 6:45 PM

To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: (b)(6) Halley Petersen-Jobsis (b)(6) Lynn Goldman (b)(6)
Subject: Re: on behalf of Dr. Anthony Fauci

Thank you, Patty. We will add the title to our agenda.

Is it okay to use Dr. Fauci's name in our promotion of this event?

Halley

On Thu, May 10, 2018 at 2:26 PM, Conrad, Patricia (NIH/NIAID) [E] (b)(6) wrote:

All:

Below is the title for Dr. Fauci's talk. Please let me know if you need anything else at this time.

AMERICAN OVERSIGHT

"Arboviruses in the Americas: Emerging and Re-emerging Threats."

Patricia L. Conrad

Special Assistant to the Director

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From: Petersen-Jobsis, Halley (b)(6)
Sent: Tuesday, May 08, 2018 10:27 AM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: (b)(6) Halley Petersen-Jobsis (b)(6) Lynn Goldman (b)(6)
Subject: Re: on behalf of Dr. Anthony Fauci

Hi Patty-

I'm coordinating the meeting, and I'm happy to help fill in the blanks. let me know if you'd like to schedule a call, or follow up via email with your questions.

Halley

On Tue, May 8, 2018 at 10:15 AM, Conrad, Patricia (NIH/NIAID) [E] (b)(6) wrote:

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Thank you for the detailed answers – this is very helpful. I will follow up with Halley – safe travels back to WDC.

Best,

-patty

Patricia L. Conrad

Special Assistant to the Director

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From: Jon Andrus (b)(6)
Sent: Tuesday, May 08, 2018 10:09 AM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Halley Petersen-Jobsis (b)(6) Lynn Goldman (b)(6)

Subject: Fwd: on behalf of Dr. Anthony Fauci

Dear Pat,

I just received your message. I am currently in (b)(6) doing work for the global measles rubella elimination initiative.

Please see my responses in parentheses to your questions below. I am copying Halley Petersen-Jobsis from the GWU who will know the exact name of the conference hall at the Milken

AMERICAN OVERSIGHT

Institute for Global Health on George Washington Circle, next to the GWU Hospital. She will also send the agenda and be your prime logistical contact.

Thanks for coordinating the visit of Dr. Fauci. I am looking forward to seeing him, I know Lynn is too!

Also, do not hesitate to contact me on my cell (b)(6) if you need any additional assistance. I will be back in WDC the evening of 17 May.

Please confirm that you received this. Connectivity is occasionally an issue.

All the best,

Jon

---------- Forwarded message ----------
From: **Lynn Goldman** (b)(6)
Date: Tue, May 8, 2018 at 8:18 PM
Subject: Fwd: on behalf of Dr. Anthony Fauci
To: Jon Andrus (b)(6) Jon Andrus (b)(6) Jon Andrus (b)(6)
Cc: Natasha Nathan (b)(6)

Dear Jon,

The NIAID is sending out a search party to find you to respond to their email about the arbovirus meeting. Please confirm that you have received this/can get back to them.

AMERICAN OVERSIGHT

Truly yours,

Lynn

---------- Forwarded message ----------
From: **Conrad, Patricia (NIH/NIAID) [E]** (b)(6)
Date: Tue, May 8, 2018 at 9:13 AM
Subject: on behalf of Dr. Anthony Fauci
To: (b)(6) (b)(6)

Good morning:

I am hoping you can help me with the below email. Can you please direct me to the conference coordinator for the below lecture. We have not heard back from Dr. Andrus.

Thank you.

Patricia L. Conrad

Public Health Analyst and

Special Assistant to the Director

National Institute of Allergy and Infectious Diseases

The National Institutes of Health

31 Center Drive, MSC 2520 - Room 7A03

Bethesda, Maryland 20892

(b)(6)

301-496-4409 fax

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Monday, May 07, 2018 11:40 AM
To: (b)(6) (b)(6)
Subject: RE: Arbovirus meeting at GWU on 5-6 June

Good morning Dr. Andrus:

I am touching base to get more detail on your event that Dr. Fauci will speak at on June 5th. We would like to prepare his remarks. Our questions include:

1. Exact time for the lecture – we are holding that morning. (8:45 am)
2. Room and building location for the event (See above. Halley will provide name.)
3. How long you would like Dr. Fauci to speak for and if there will be a q and a after (We would like Dr. Fauci to speak for 25 minutes with 5 minutes to field a few questions. I know he is busy, so he could depart after a few questions. I will be moderating. We are planning to have webcast - Halley can confirm)
4. Who is in the audience and how many? So that we can tailor his remarks (The key audience is a group of 25 global experts, mostly from Latin American and the Caribbean, who will be writing a white paper to inform policy on the prevention and control of Aedes transmitted Arboviruses in Latin America and the Caribbean. PAHO is fully engaged and appreciates this effort. In addition, there will be public health experts attending from the

AMERICAN OVERSIGHT

PAHO general staff, other academics from LAC and the US, and other public health partners in the WDC area. Finally, the room of 220 occupancy will also have in attendance graduate students and other interested faculty.)

5. Please confirm that slides are appropriate – Dr. Fauci will use powerpoint. We ask that you provide a PC laptop at the podium so that he can closely see and advance his own slides (Yes, PPT presentation is welcomed and we will be sure he access to a laptop at the lectern.)
6. Is there a detailed agenda you can send with who else will be speaking (I will ask Halley by copy of this to her to send you the detailed agenda, since I am (b)(6) and will not be returning until 17 May.)
7. Please send the onsite logistical contacts. (Halley will be your prime logistical contact)

Thank you and I look forward to hearing from you.

Best,

-patty

Patricia L. Conrad

Special Assistant to the Director

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Tuesday, January 09, 2018 3:55 PM
To: (b)(6) (b)(6)
Subject: FW: Arbovirus meeting at GWU on 5-6 June

Dr. Andrus:

Dr. Fauci asked that I respond to your email on his behalf – he wanted me to thank you for contacting him. He would be happy to deliver the keynote at your meeting on June 5th. I will be the point of contact for his logistics. Please let me know is you need anything else at this time. Otherwise, we can just touch base closer to the event date.

Best,

-patty

Patricia L. Conrad

Special Assistant to the Director

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Begin forwarded message:

AMERICAN OVERSIGHT

From: Jon Andrus (b)(6)
Date: January 9, 2018 at 10:59:11 AM EST
To: (b)(6)
Subject: Arbovirus meeting at GWU on 5-6 June
Reply-To: (b)(6)

Dear Tony,

Hope all is well.

Dean Lynn Goldman of GWU and I are organizing a scientific symposium on prevention and control of arboviruses 5-6 June at GWU in WDC. I am writing to see if you would be willing to provide the keynote address.

When I was Deputy Director of PAHO, you gave an outstanding presentation in celebration of the lifetime contribution to global health of Dr. Ciro de Quadros. We organized that event a few months before he died. At the time he whispered in my ear, "Just what the doctor ordered." For that I am eternally grateful for your contribution. I know Ciro was. I also remember distinctly the tremendous help you provided us when Dr. John LaMontagne died in Mexico City on his way to the PAHO TAG meeting, taking my calls at all hours to assist the family.

It would be an honor and pleasure to be able to work with you again on an important issue if you are available to give the keynote speech the morning of 5 June. Thanks for your time and consideration.

All the best,

Jon

Jon Kim Andrus, MD

Adjunct Professor Global Health

Milken Institute of Public Health

George Washington University

Adjoint Professor and Director of the Department of Vaccines and Immunization

Center for Global Health

University of Colorado

AMERICAN OVERSIGHT

--

Lynn R. Goldman, MD, MS, MPH
Michael and Lori Milken Dean, Milken Institute School of Public Health,

George Washington University

(b)(6)

--

Halley Petersen-Jöbsis MPH | Academic Program Manager and Academic Advisor | Department of Global Health

Milken Institute School of Public Health | The George Washington University

950 New Hampshire Ave NW | 4th Floor | Washington, DC 20052

(b)(6)

(b)(6)

--

Halley Petersen-Jöbsis MPH | Academic Program Manager and Academic Advisor | Department of Global Health

Milken Institute School of Public Health | The George Washington University

AMERICAN OVERSIGHT

950 New Hampshire Ave NW | 4th Floor | Washington, DC 20052

(b)(6)

(b)(6)

--

Halley Petersen-Jöbsis MPH | Academic Program Manager and Academic Advisor | Department of Global Health

Milken Institute School of Public Health | The George Washington University

950 New Hampshire Ave NW | 4th Floor | Washington, DC 20052

(b)(6)

(b)(6)

--

Halley Petersen-Jöbsis MPH | Academic Program Manager and Academic Advisor | Department of Global Health

Milken Institute School of Public Health | The George Washington University

950 New Hampshire Ave NW | 4th Floor | Washington, DC 20052

(b)(6)

(b)(6)

AMERICAN OVERSIGHT

--

Halley Petersen-Jöbsis MPH | Academic Program Manager and Academic Advisor | Department of Global Health

Milken Institute School of Public Health | The George Washington University

950 New Hampshire Ave NW | 4th Floor | Washington, DC 20052

(b)(6)

(b)(6)

AMERICAN OVERSIGHT

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Wed, 16 May 2018 18:41:53 +0000
To: Petersen-Jobsis, Halley
Cc: Vance, Meaghan (NIH/NIAID) [C]
Subject: RE: on behalf of Dr. Anthony Fauci

Halley –

Dr. Fauci will do the interview. He will need to do it immediately after his lecture. Since his lecture is 8:45 am – 9:15 am – I will hold 9:30 -9:45 am.

Please send me the contact name and cell number for this. It would be great to get the questions a week in advance.

thx

Patricia L. Conrad
Special Assistant to the Director

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From: Petersen-Jobsis, Halley (b)(6)
Sent: Wednesday, May 16, 2018 1:37 PM
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Subject: Re: on behalf of Dr. Anthony Fauci

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Halley

Michael and Lori Milken Dean Lynn R. Goldman

and

Professor of Global Health Jon Andrus

The George Washington University Milken Institute School of Public Health

Cordially invite you to a special event

Prevention and Control of Emerging Arbovirus Infections in Latin America and the Caribbean

An internationally recognized panel of Arbovirus experts will discuss status, progress and challenges around the management, diagnostics, surveillance, and prevention and control strategies of emerging Arbovirus infections, to inform policy decisions in Latin America and the Caribbean.

Keynote presentation by **Dr. Anthony Fauci**

Global Arbovirus Disease Burden: Challenges for Prevention and Control

Plenary sessions include:

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- Overview of vaccine research and development: Dengue, Yellow Fever, Zika, and Chikungunya
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- Considerations in vaccine delivery strategies

AMERICAN OVERSIGHT

June 5, 2018

8:30 am - 5:30 pm | Auditorium

Milken Institute School of Public Health

950 New Hampshire Ave, NW

Washington, DC

RSVP

On Sat, May 12, 2018 at 7:03 AM, Conrad, Patricia (NIH/NIAID) [E] (b)(6) wrote:
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Sent from my iPhone

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H

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Patricia L. Conrad
Special Assistant to the Director

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From: Petersen-Jobsis, Halley (b)(6)
Sent: Thursday, May 10, 2018 6:45 PM

To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: (b)(6) Halley Petersen-Jobsis (b)(6) Lynn Goldman (b)(6)
Subject: Re: on behalf of Dr. Anthony Fauci

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Is it okay to use Dr. Fauci's name in our promotion of this event?

Halley

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"Arboviruses in the Americas: Emerging and Re-emerging Threats."

Patricia L. Conrad
Special Assistant to the Director

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From: Petersen-Jobsis, Halley (b)(6)
Sent: Tuesday, May 08, 2018 10:27 AM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: (b)(6) Halley Petersen-Jobsis (b)(6) Lynn Goldman (b)(6)
Subject: Re: on behalf of Dr. Anthony Fauci

Hi Patty-

I'm coordinating the meeting, and I'm happy to help fill in the blanks. let me know if you'd like to schedule a call, or follow up via email with your questions.

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Halley

On Tue, May 8, 2018 at 10:15 AM, Conrad, Patricia (NIH/NIAID) [E] (b)(6) wrote:

Thank you for the detailed answers – this is very helpful. I will follow up with Halley – safe travels back to WDC.

Best,
-patty

Patricia L. Conrad
Special Assistant to the Director

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From: Jon Andrus (b)(6)
Sent: Tuesday, May 08, 2018 10:09 AM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Halley Petersen-Jobsis (b)(6) Lynn Goldman (b)(6)

Subject: Fwd: on behalf of Dr. Anthony Fauci

Dear Pat,

I just received your message. I am currently in (b)(6) doing work for the global measles rubella elimination initiative.

Please see my responses in parentheses to your questions below. I am copying Halley Petersen-Jobsis from the GWU who will know the exact name of the conference hall at the Milken Institute for Global Health on George Washington Circle, next to the GWU Hospital. She will also send the agenda and be your prime logistical contact.

Thanks for coordinating the visit of Dr. Fauci. I am looking forward to seeing him, I know Lynn is too!

Also, do not hesitate to contact me on my cell (b)(6) if you need any additional assistance. I will be back in WDC the evening of 17 May.

Please confirm that you received this. Connectivity is occasionally an issue.

All the best,

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Jon

---------- Forwarded message ----------
From: **Lynn Goldman** (b)(6)
Date: Tue, May 8, 2018 at 8:18 PM
Subject: Fwd: on behalf of Dr. Anthony Fauci
To: Jon Andrus (b)(6) Jon Andrus (b)(6) Jon Andrus (b)(6)
Cc: Natasha Nathan (b)(6)

Dear Jon,

The NIAID is sending out a search party to find you to respond to their email about the arbovirus meeting. Please confirm that you have received this/can get back to them.

Truly yours,
Lynn
---------- Forwarded message ----------
From: **Conrad, Patricia (NIH/NIAID) [E]** (b)(6)
Date: Tue, May 8, 2018 at 9:13 AM
Subject: on behalf of Dr. Anthony Fauci
To: (b)(6) (b)(6)

Good morning:

I am hoping you can help me with the below email. Can you please direct me to the conference coordinator for the below lecture. We have not heard back from Dr. Andrus.

Thank you.

Patricia L. Conrad
Public Health Analyst and
Special Assistant to the Director
National Institute of Allergy and Infectious Diseases
The National Institutes of Health
31 Center Drive, MSC 2520 - Room 7A03
Bethesda, Maryland 20892
(b)(6)
301-496-4409 fax

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Monday, May 07, 2018 11:40 AM
To: (b)(6) (b)(6)
Subject: RE: Arbovirus meeting at GWU on 5-6 June

Good morning Dr. Andrus:

I am touching base to get more detail on your event that Dr. Fauci will speak at on June 5th. We would like to prepare his remarks. Our questions include:

1. Exact time for the lecture – we are holding that morning. (8:45 am)
2. Room and building location for the event (See above. Halley will provide name.)
3. How long you would like Dr. Fauci to speak for and if there will be a q and a after (We would like Dr. Fauci to speak for 25 minutes with 5 minutes to field a few questions. I know he is busy, so he could depart after a few questions. I will be moderating. We are planning to have webcast - Halley can confirm)
4. Who is in the audience and how many? So that we can tailor his remarks (The key audience is a group of 25 global experts, mostly from Latin American and the Caribbean, who will be writing a white paper to inform policy on the prevention and control of Aedes transmitted Arboviruses in Latin America and the Caribbean. PAHO is fully engaged and appreciates this effort. In addition, there will be public health experts attending from the

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PAHO general staff, other academics from LAC and the US, and other public health partners in the WDC area. Finally, the room of 220 occupancy will also have in attendance graduate students and other interested faculty.)

5. Please confirm that slides are appropriate – Dr. Fauci will use powerpoint. We ask that you provide a PC laptop at the podium so that he can closely see and advance his own slides (Yes, PPT presentation is welcomed and we will be sure he access to a laptop at the lectern.)
6. Is there a detailed agenda you can send with who else will be speaking (I will ask Halley by copy of this to her to send you the detailed agenda, since I am (b)(6) and will not be returning until 17 May.)
7. Please send the onsite logistical contacts. (Halley will be your prime logistical contact)

Thank you and I look forward to hearing from you.

Best,
-patty

Patricia L. Conrad
Special Assistant to the Director

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Tuesday, January 09, 2018 3:55 PM
To: (b)(6) (b)(6)
Subject: FW: Arbovirus meeting at GWU on 5-6 June

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Dr. Andrus:

Dr. Fauci asked that I respond to your email on his behalf – he wanted me to thank you for contacting him. He would be happy to deliver the keynote at your meeting on June 5th. I will be the point of contact for his logistics. Please let me know is you need anything else at this time. Otherwise, we can just touch base closer to the event date.

Best,
-patty

Patricia L. Conrad
Special Assistant to the Director

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Begin forwarded message:

From: Jon Andrus (b)(6)
Date: January 9, 2018 at 10:59:11 AM EST
To: (b)(6)
Subject: Arbovirus meeting at GWU on 5-6 June
Reply-To: (b)(6)

Dear Tony,

Hope all is well.

Dean Lynn Goldman of GWU and I are organizing a scientific symposium on prevention and control of arboviruses 5-6 June at GWU in WDC. I am writing to see if you would be willing to provide the keynote address.

When I was Deputy Director of PAHO, you gave an outstanding presentation in celebration of the lifetime contribution to global health of Dr. Ciro de Quadros. We organized that event a few months before he died. At the time he whispered in my ear, "Just what the doctor ordered." For that I am eternally grateful for your contribution. I know Ciro was. I also remember distinctly the tremendous help you provided us when Dr. John LaMontagne died in Mexico City on his way to the PAHO TAG meeting, taking my calls at all hours to assist the family.

It would be an honor and pleasure to be able to work with you again on an important issue if you are available to give the keynote speech the morning of 5 June. Thanks for your time and consideration.

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All the best,

Jon

Jon Kim Andrus, MD
Adjunct Professor Global Health
Milken Institute of Public Health
George Washington University

Adjoint Professor and Director of the Department of Vaccines and Immunization
Center for Global Health
University of Colorado

--

Lynn R. Goldman, MD, MS, MPH
Michael and Lori Milken Dean, Milken Institute School of Public Health,
George Washington University
(b)(6)

--

Halley Petersen-Jöbsis MPH | Academic Program Manager and Academic Advisor | Department of Global Health
Milken Institute School of Public Health | The George Washington University
950 New Hampshire Ave NW | 4th Floor | Washington, DC 20052
(b)(6)
(b)(6)

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Halley Petersen-Jöbsis MPH | Academic Program Manager and Academic Advisor | Department of Global Health
Milken Institute School of Public Health | The George Washington University
950 New Hampshire Ave NW | 4th Floor | Washington, DC 20052
(b)(6)
(b)(6)

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Halley Petersen-Jöbsis MPH | Academic Program Manager and Academic Advisor | Department of Global Health

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950 New Hampshire Ave NW | 4th Floor | Washington, DC 20052

(b)(6)

(b)(6)

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From: Golden, Matthew
Sent: Wed, 16 May 2018 15:03:58 -0400
To: Conrad, Patricia (NIH/NIAID) [E]
Cc: Petersen-Jobsis, Halley
Subject: Re: Dr. Fauci interview at GW SPH on 6/5

Certainly, my number is (b)(6)

I will be in touch in the coming days with questions and any additional details.

Thanks,
Matthew

On Wed, May 16, 2018 at 2:59 PM, Conrad, Patricia (NIH/NIAID) [E]
(b)(6) wrote:

Thank you. I will just need both of your cell numbers for the day of the event – just in case we have any last minute issues but I don't expect any.

Many thanks,

-patty

-

Patricia L. Conrad

Special Assistant to the Director

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From: Petersen-Jobsis, Halley (b)(6)
Sent: Wednesday, May 16, 2018 2:53 PM
To: Matthew Golden (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Subject: Dr. Fauci interview at GW SPH on 6/5

Matthew and Patty-

This email is to put you in touch re: Dr. Fauci's interview following his keynote address on arboviruses. Dr. Fauci has graciously agreed to a 15 minute interview immediately following his talk.

Patty Conrad is our contact in Dr. Fauci's office. She has scheduled Dr. Fauci to be interviewed from 9:30-9:45am on 6/5.

Matthew Golden will be the primary contact at GWSPH for this interview. He will send over the questions 1 week in advance (as requested by Patty) and will provide any other information that Dr. Fauci needs prior to the interview.

Let me know if you have any questions!

Halley

--

Halley Petersen-Jöbsis MPH | Academic Program Manager and Academic Advisor | Department of Global Health

Milken Institute School of Public Health | The George Washington University

950 New Hampshire Ave NW | 4th Floor | Washington, DC 20052

(b)(6)

(b)(6)

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Matthew Golden
Executive Director, Communications
Milken Institute School of Public Health
The George Washington University
(b)(6)
(b)(6)

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From: Schwetz, Tara (NIH/NIAID) [E]
Sent: Wed, 16 May 2018 21:43:14 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Folkers, Greg (NIH/NIAID) [E];Conrad, Patricia (NIH/NIAID) [E];Silver, Cheryl (NIH/NIAID) [E];Ben-Ari, Elia (NIH/NIAID) [C]
Subject: Round 1 FY18 Science Advances
Attachments: 2018-05-14_NIAID FY 2018 Round 1 Science Advances_rev2[1].docx

Tony,

It's that time of year again – when you select the recent papers from NIAID-funded projects that can be featured as part of the Institute's "top ten science advances."

Each NIAID Division was invited to submit its top five advances published from October 1, 2018 to March 31, 2018. The complete list of advances suggested by the Divisions is attached for your review. Please note that DIR submitted two companion papers to be considered as a single advance. We also included Malcolm Martin's paper on SHIV (with the approval of DIR), which was recently included in the HIV/AIDS advances for OAR.

We ask that you please select the ten advances that you feel represent the Institute's most important research with potential public health impact. Thank you, and please do let us know if you need anything else.

Best,

Tara A. Schwetz, PhD
Branch Chief, SPEB/NIAID
A: Building 31, Room 7A46D
P: (b)(6) | **M:** (b)(6)

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NIAID Science Advances – FY 2018 Q1-Q2

Publication	PI/Affiliation	Journal	NIAID News Release	Division	Ranking
(b)(5)					
		Medicine	Hepatology	Yes	DAIT
(b)(5)					

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(b)(5)

(b)(5)

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(b)(5)

(b)(5)

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(b)(5)

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Wed, 16 May 2018 20:57:41 +0000
To: NIAID OD AM
Subject: FW: An invitation as a keynote speaker at a University of Giessen and Marburg Lung Center/Max Planck Institute symposium on "RNA Viruses" (19-21 September, 2018)
Attachments: Invitation-Anthony_Fauci_2018_RNA-Viruses-Symposium.pdf

Let us discuss. Almost certainly a no.

From: Morty, Rory (b)(6)
Sent: Wednesday, May 16, 2018 10:14 AM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: An invitation as a keynote speaker at a University of Giessen and Marburg Lung Center/Max Planck Institute symposium on "RNA Viruses" (19-21 September, 2018)

[An original, signed invitation on official stationary is attached in PDF format]

Bad Nauheim, Germany; Wednesday, 16th May 2018

Dr. Anthony S. Fauci
Director
National Institute for Allergy & Infectious Diseases
National Institute of Health
31 Center Drive
Bethesda MD 20814
UNITED STATES OF AMERICA

-

Dear Dr. Fauci

Invitation as a keynote speaker at a University of Giessen and Marburg Lung Center and Max Planck Institute symposium on "RNA viruses" on 19-21 September 2018.

I am writing to you today to invite you to deliver a keynote address at an upcoming joint symposium of the University of Giessen and Marburg Lung Center and the Max Planck Institute for Heart and Lung Research, in Germany.

Our meeting addresses the topic of "RNA viruses", and is a symposium of two funding consortia: one supporting basic research into RNA viruses, focusing on filoviruses, hepatitis, and influenza (http://www.sfb1021.de/home.html); while the second supports clinical research into virus-induced lung injury (http://www.kfo309.de/). The meeting takes place on 19-21 September, 2018, in Marburg (home of the first Marburg fever outbreak), near Frankfurt, Germany; in an historical "outbreak" city, and prioritises our younger "next generation" of infectious disease specialists, promises high quality translational and basic science, and offers full financial support for all of your travel, including business class flights. We intended hosting a pre-dinner keynote address on the evening of 19 September 2018, and I would like to solicit your interest and

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availability to join us then? Your fundamental discoveries on innate immunity are clearly most relevant to out meeting! If this might be a possibility for you, I would be very happy to provide you with any information that you may require.

Should you be able to join us, for part or all of our symposium, we would be delighted.

I look very forward to hearing from you!

Yours very sincerely,

Rory E. Morty
Co-organiser

Rory E. Morty
Department of Lung Development and Remodelling
Max Planck Institute for Heart and Lung Research
Parkstrasse 1, D-61231 Bad Nauheim, GERMANY

Department of Internal Medicine (Pulmonology)
University Hospital Giessen and Marburg
Aulweg 123, D-35392 Giessen, GERMANY

Tel: (b)(6) (direct)
Tel: (b)(6) (secretary: research)
Tel: (b)(6) (secretary: clinical)
Fax: +49 6032 705 471
Email: (b)(6)

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Max-Planck-Institut
für Herz- und Lungenforschung
W.G. Kerckhoff-Institut

Max-Planck-Institut für Herz- und Lungenforschung
Parkstraße 1 D-61231 Bad Nauheim

Dr. Anthony S. Fauci
Director
National Institute for Allergy & Infectious Diseases
National Institute of Health
31 Center Drive
Bethesda MD 20814
UNITED STATES OF AMERICA

Ihr Zeichen: nicht zutreffend
Ihre Nachricht vom: nicht zutreffend

Datum: 16 May 2018

Dear Dr. Fauci

Invitation as a keynote speaker at a University of Giessen and Marburg Lung Center and Max Planck Institute symposium on "RNA viruses" on 19-21 September 2018.

I am writing to you today to invite you to deliver a keynote address at an upcoming joint symposium of the University of Giessen and Marburg Lung Center and the Max Planck Institute for Heart and Lung Research, in Germany. Our meeting addresses the topic of "RNA viruses", and is a symposium of two funding consortia: one supporting basic research into RNA viruses, focusing on filoviruses, hepatitis, and influenza (http://www.sfb1021.de/home.html); while the second supports clinical research into virus-induced lung injury (http://www.kfo309.de/). The meeting takes place on 19-21 September, 2018, in Marburg (home of the first Marburg fever outbreak), near Frankfurt, Germany; in an historical "outbreak" city, and prioritises our younger "next generation" of infectious disease specialists, promises high quality translational and basic science, and offers full financial support for all of your travel, including business class flights. We intended hosting a pre-dinner keynote address on the evening of 19 September 2018, and I would like to solicit your interest and availability to join us then? Your fundamental discoveries on innate immunity are clearly most relevant to out meeting! If this might be a possibility for you, I would be very happy to provide you with any information that you may require. Should you be able to join us, for part or all of our event, we would be delighted. I look very forward to hearing from you!

Yours very sincerely,

(b)(6)

Rory E. Morty
(Co-organiser)

Max-Planck-Institut für Herz- und Lungenforschung W.G. Kerckhoff-Institut
Abteilung IV: Entwicklung und Umbau der Lunge
Dr Rory E. Morty · Parkstraße 1 · D-61231 Bad Nauheim
(b)(6) · Tel: (b)(6) · Fax: +49 (0)6032. 705-471
www.mpi-bn.mpg.de · info@mpi-bn.mpg.de

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From: Shapiro, Neil (NIH/OD) [E]
Sent: Wed, 16 May 2018 20:11:45 +0000
To: Collins, Francis (NIH/OD) [E];Fauci, Anthony (NIH/NIAID) [E];Hodes, Richard (NIH/NIA) [E];Koroshetz, Walter (NIH/NINDS) [E];Sharpless, Norman (NIH/NCI) [E];Volkow, Nora (NIH/NIDA) [E]
Cc: Tabak, Lawrence (NIH/OD) [E];Johnson, Alfred (NIH/OD) [E];Wolinetz, Carrie (NIH/OD) [E];Hallett, Adrienne (NIH/OD) [E]
Subject: RCDC Levels
Attachments: RCDC FY 2017 Actuals and FY 2018-2019 Estimates.pdf

Folks,

The attached file is an advance copy of the most recent actual and estimated annual funding data (FY 2017 – FY 2019) for published RCDC categories. This information will be uploaded to the RePORT web site later this week. The update includes FY 2017 actuals, FY 2018 estimated at the Operating Plan level, and FY 2019 estimated at the President's Budget level (consistent with the Congressional Justification). This was provided to the House and Senate appropriations staff last night, so you can use the numbers at the hearing if appropriate.

Neil

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RCDC FY 2017 Actuals & FY 2018-2019 Estimates

(Rounded to millions)

Description	2017 Actual	2018 Estimate	2019 Estimate
Acquired Cognitive Impairment	1,560	(b)(5)	
Acute Respiratory Distress Syndrome	107		
Adolescent Sexual Activity	99		
Agent Orange & Dioxin	11		
Aging	3,572		
Alcoholism, Alcohol Use and Health	500		
Allergic Rhinitis (Hay Fever)	6		
ALS	78		
Alzheimer's Disease	1,361		
Alzheimer's Disease including Alzheimer's Disease Related Dementias (AD/ADRD)	1,423		
Alzheimer's Disease Related Dementias (ADRD)	249		
American Indian or Alaska Native	181		
Anorexia	10		
Anthrax	50		
Antimicrobial Resistance	470		
Anxiety Disorders	190		
Aphasia	34		
Arctic	20		
Arthritis	240		
Assistive Technology	299		
Asthma	286		
Ataxia Telangiectasia	10		
Atherosclerosis	426		
Attention Deficit Disorder (ADD)	54		
Autism	245		
Autoimmune Disease	934		
Back Pain	30		
Basic Behavioral and Social Science	1,993		
Batten Disease	5		
Behavioral and Social Science	4,547		
Biodefense	1,964		
Bioengineering	4,106		
Biomedical Imaging	2,083		
Biotechnology	6,556		
Bipolar Disorder	88		
Brain Cancer	332		
Brain Disorders	5,156		
Breast Cancer	689		
Breastfeeding, Lactation and Breast Milk	92		
Burden of Illness	109		
Cachexia	20		
Cancer	5,980		
Cancer Genomics	907		
Cannabidiol Research	15		
Cannabinoid Research	140		
Cardiovascular	2,197		
Caregiving Research	87		
Cerebral Palsy	26		
Cerebrovascular	610		
Cervical Cancer	114		
Charcot-Marie-Tooth Disease	10		
Child Abuse and Neglect Research	29		
Childhood Leukemia	177		
Chronic Fatigue Syndrome (ME/CFS)	15		
Chronic Liver Disease and Cirrhosis	285		
Chronic Obstructive Pulmonary Disease	100		
Climate Change	7		
Climate-Related Exposures and Conditions	193		
Clinical Research	12,695		
Clinical Trials and Supportive Activities	3,775		
Colo-Rectal Cancer	270		
Comparative Effectiveness Research	685		
Complementary and Alternative Medicine	376		
Conditions Affecting the Embryonic and Fetal Periods	180		
Congenital Heart Disease	119		
Congenital Muscular Dystrophy	9		
Congenital Structural Anomalies	474		
Contraception/Reproduction	437		
Cooley's Anemia	18		
Cost Effectiveness Research	223		
Crohn's Disease	67		
Cystic Fibrosis	91		
Dementia	1,470		
Dental/Oral and Craniofacial Disease	541		

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RCDC FY 2017 Actuals & FY 2018-2019 Estimates

(Rounded to millions)

Description	2017 Actual	2018 Estimate	2019 Estimate
Depression	438	(b)(5)	
Diabetes	1,108		
Dietary Supplements	296		
Diethylstilbestrol (DES)	4		
Digestive Diseases	1,881		
Digestive Diseases - (Gallbladder)	12		
Digestive Diseases - (Peptic Ulcer)	8		
Down Syndrome	35		
Drug Abuse (NIDA only)	1,071		
Duchenne/ Becker Muscular Dystrophy	30		
Dystonia	15		
Eating Disorders	32		
Eczema / Atopic Dermatitis	32		
Emergency Care	139		
Emerging Infectious Diseases	2,591		
Emphysema	27		
Endocannabinoid System Research	63		
Endocrine Disruptors	177		
Endometriosis	6		
Epilepsy	154		
Estrogen	220		
Eye Disease and Disorders of Vision	882		
Facioscapulohumeral Muscular Dystrophy	11		
Fetal Alcohol Syndrome	28		
Fibroid Tumors (Uterine)	11		
Fibromyalgia	14		
Food Allergies	79		
Foodborne Illness	105		
Fragile X Syndrome	46		
Frontotemporal Dementia (FTD)	91		
Gene Therapy	266		
Gene Therapy Clinical Trials	34		
Genetic Testing	148		
Genetics	8,501		
Global Warming Climate Change	4		
Headaches	30		
Health Disparities	3,168		
Health Effects of Household Energy Combustion	17		
Health Effects of Indoor Air Pollution	104		
Health Services	1,770		
Heart Disease	1,370		
Heart Disease - Coronary Heart Disease	444		
Hematology	1,385		
Hepatitis	306		
Hepatitis - A	4		
Hepatitis - B	42		
Hepatitis - C	114		
HIV/AIDS	3,000		
Hodgkin's Disease	12		
Homelessness	17		
Homicide and Legal Interventions	1		
HPV and/or Cervical Cancer Vaccines	59		
Human Fetal Tissue	98		
Human Genome	3,274		
Huntington's Disease	47		
Hydrocephalus	11		
Hyperbaric Oxygen	6		
Hypertension	235		
Immunization	2,382		
Infant Mortality	96		
Infectious Diseases	5,684		
Infertility	94		
Inflammatory Bowel Disease	134		
Influenza	301		
Injury (total) Accidents/Adverse Effects	496		
Injury - Childhood Injuries	60		
Injury - Trauma - (Head and Spine)	222		
Injury - Traumatic brain injury	116		
Injury - Unintentional Childhood Injury	31		
Intellectual and Developmental Disabilities (IDD)	455		
Interstitial Cystitis	10		
Kidney Disease	592		
Lead Poisoning	10		
Lewy Body Dementia	31		

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RCDC FY 2017 Actuals & FY 2018-2019 Estimates

(Rounded to millions)

Description	2017 Actual	2018 Estimate	2019 Estimate
Liver Cancer	90	(b)(5)	
Liver Disease	691		
Lung	1,718		
Lung Cancer	352		
Lupus	109		
Lyme Disease	22		
Lymphoma	266		
Macular Degeneration	100		
Major Depressive Disorder	104		
Malaria	192		
Malaria Vaccine	61		
Maternal Health	249		
Mental Health	2,717		
Mental Illness	882		
Methamphetamine	48		
Migraines	22		
Mind and Body	279		
Minority Health	2,934		
Mucopolysaccharidoses (MPS)	7		
Multiple Sclerosis	111		
Muscular Dystrophy	81		
Myasthenia Gravis	5		
Myotonic Dystrophy	11		
Nanotechnology	444		
Neck Pain	2		
Neonatal Respiratory Distress	62		
Networking and Information Technology R&D	1,149		
Neuroblastoma	53		
Neurodegenerative	2,554		
Neurofibromatosis	31		
Neurosciences	7,317		
Nutrition	1,708		
Obesity	999		
Organ Transplantation	170		
Orphan Drug	1,653		
Osteoarthritis	78		
Osteogenesis Imperfecta	13		
Osteoporosis	139		
Otitis Media	9		
Ovarian Cancer	151		
Paget's Disease	2		
Pain Conditions - Chronic	440		
Pain Research	516		
Pancreatic Cancer	199		
Parkinson's Disease	168		
Patient Safety	1,099		
Pediatric	4,176		
Pediatric AIDS	179		
Pediatric Cancer	433		
Pediatric Cardiomyopathy	30		
Pediatric Research Initiative	568		
Pelvic Inflammatory Disease	2		
Perinatal Period - Conditions Originating in Perinatal Period	616		
Peripheral Neuropathy	121		
Physical Activity	421		
Physical Rehabilitation	146		
Pick's Disease	8		
Pneumonia	114		
Pneumonia & Influenza	408		
Polycystic Kidney Disease	29		
Post-Traumatic Stress Disorder (PTSD)	101		
Precision Medicine	1,152		
Pregnancy	319		
Prescription Drug Abuse	69		
Preterm, Low Birth Weight and Health of the Newborn	291		
Prevention	8,052		
Prostate Cancer	239		
Psoriasis	17		
Radiation Oncology	313		
Rare Diseases	4,613		
Regenerative Medicine	939		
Rehabilitation	565		
Rett Syndrome	15		
Rheumatoid Arthritis	93		

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RCDC FY 2017 Actuals & FY 2018-2019 Estimates

(Rounded to millions)

Description	2017 Actual	2018 Estimate	2019 Estimate
Rural Health	259	(b)(5)	
Sarcopenia	19		
Schizophrenia	243		
Scleroderma	17		
Screening And Brief Intervention For Substance Abuse	38		
Septicemia	125		
Serious Mental Illness	402		
Sexual and Gender Minorities (SGM/LGBT*)	233		
Sexually Transmitted Diseases/Herpes	322		
Sickle Cell Disease	109		
Sleep Research	327		
Small Pox	57		
Smoking and Health	293		
Spina Bifida	13		
Spinal Cord Injury	77		
Spinal Muscular Atrophy	11		
Stem Cell Research	1,646		
Stem Cell Research - Embryonic - Human	252		
Stem Cell Research - Embryonic - Non-Human	129		
Stem Cell Research - Induced Pluripotent Stem Cell	421		
Stem Cell Research - Induced Pluripotent Stem Cell - Human	382		
Stem Cell Research - Induced Pluripotent Stem Cell - Non-Human	59		
Stem Cell Research - Nonembryonic - Human	484		
Stem Cell Research - Nonembryonic - Non-Human	704		
Stem Cell Research - Umbilical Cord Blood/ Placenta	40		
Stem Cell Research - Umbilical Cord Blood/ Placenta - Human	35		
Stem Cell Research - Umbilical Cord Blood/ Placenta - Non-Human	6		
Stroke	331		
Substance Abuse	1,636		
Substance Abuse Prevention	53		
Sudden Infant Death Syndrome	13		
Suicide	68		
Suicide Prevention	35		
Teenage Pregnancy	14		
Temporomandibular Muscle/Joint Disorder (TMJD)	13		
Therapeutic Cannabinoid Research	36		
Tobacco	293		
Topical Microbicides	91		
Tourette Syndrome	6		
Transmissible Spongiform Encephalopathy (TSE)	32		
Transplantation	606		
Tuberculosis	347		
Tuberculosis Vaccine	29		
Tuberous Sclerosis	21		
Underage Drinking	66		
Underage Drinking - Prevention & Treatment (NIAAA Only)	51		
Urologic Diseases	515		
Usher Syndrome	18		
Uterine Cancer	45		
Vaccine Related	1,823		
Vaccine Related (AIDS)	562		
Vaginal Cancer	5		
Valley Fever	7		
Vascular Cognitive Impairment/Dementia	130		
Vector-Borne Diseases	557		
Violence Against Women	30		
Violence Research	115		
Vulvodynia	1		
West Nile Virus	42		
Women's Health	4,769		
Youth Violence	50		
Youth Violence Prevention	21		

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Wed, 16 May 2018 16:00:08 +0000
To: Fauci, Anthony (NIH/NIAID) [E];Handley, Gray (NIH/NIAID) [E]
Subject: Pre-brief for Serum Institute Leadership visit
Attachments: RE: Meeting Request - Serum Institute Leadership, Briefing documents for Serum Inst Visit June 5, 2018; Pre-brief, May 31, 2018

Greg, I think a 10 minute overview presentation would be a great way to start the meeting.

He did similar when he met with the CEO/son, Mr. Poonawalla last year. The meeting went very well; they hit it off. With the founder/father, Dr. Poonawalla, at this meeting, making a similar start would be helpful to kick of a general discussion.

He might especially focus on intramural (VRC and DIR) efforts to discover and develop vaccines and then link to industry to carry them forward. Serum has licensed Whitehead live attenuated dengue vaccine and will undertake phase III trials probably in 2019. They are interested in VRC vaccines as well.

Let me know if you need anything. G

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From: Williams, Nekisha (NIH/NIAID) [C]
Sent: Wed, 16 May 2018 15:55:58 +0000
To: Vance, Meaghan (NIH/NIAID) [C]
Cc: Handley, Gray (NIH/NIAID) [E];Conrad, Patricia (NIH/NIAID) [E]
Subject: RE: Meeting Request - Serum Institute Leadership

Also I will advise Dr. Meegan that his attendance for the June 5th visit is needed!

Thanks!

From: Vance, Meaghan (NIH/NIAID) [C]
Sent: Wednesday, May 16, 2018 11:52 AM
To: Williams, Nekisha (NIH/NIAID) [C] (b)(6)
Cc: Handley, Gray (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: RE: Meeting Request - Serum Institute Leadership

Nekisha-

We will schedule the briefing with Gray, but would like for Dr. Meegan to attend the actual meeting on June 5th

For the pre-brief, ASF has the following availability

May 31st 11:30-2:30pm

From: Williams, Nekisha (NIH/NIAID) [C]
Sent: Tuesday, May 15, 2018 10:55 AM
To: Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Cc: Handley, Gray (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: RE: Meeting Request - Serum Institute Leadership

Yes, he can attend the meeting if need be!

Thanks,

Nekisha

From: Vance, Meaghan (NIH/NIAID) [C]
Sent: Tuesday, May 15, 2018 10:52 AM
To: Williams, Nekisha (NIH/NIAID) [C] (b)(6)
Cc: Handley, Gray (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: RE: Meeting Request - Serum Institute Leadership

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Okay – so Dr. Meegan will be the one attending the actual meeting on June 5th?

From: Williams, Nekisha (NIH/NIAID) [C]
Sent: Tuesday, May 15, 2018 10:51 AM
To: Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Cc: Handley, Gray (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: RE: Meeting Request - Serum Institute Leadership

No. He will be in (b)(6) during the June 5th meeting.

Thanks,

Nekisha

From: Vance, Meaghan (NIH/NIAID) [C]
Sent: Tuesday, May 15, 2018 10:49 AM
To: Williams, Nekisha (NIH/NIAID) [C] (b)(6)
Cc: Handley, Gray (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: RE: Meeting Request - Serum Institute Leadership

Will gray be available for the meeting on June 5th?

From: Williams, Nekisha (NIH/NIAID) [C]
Sent: Tuesday, May 15, 2018 10:48 AM
To: Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Cc: Handley, Gray (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: RE: Meeting Request - Serum Institute Leadership

I missed the email so my apologies!

Gray will not be in the states on June 5th he will be in (b)(6) attending site visits. Dr. Meegan can do the pre brief on June 4th in Gray's absence.

Thanks Meaghan,

Nekisha

From: Vance, Meaghan (NIH/NIAID) [C]
Sent: Tuesday, May 15, 2018 10:42 AM
To: Williams, Nekisha (NIH/NIAID) [C] (b)(6)
Subject: RE: Meeting Request - Serum Institute Leadership

Hi Nekisha:

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We sent the attached email – sorry if I missed the reply…but we had a few questions…

If we can't do it before you leave, who would be available to brief ASF on June 4th?

Also, will you be here on June 5th to attend the actual meeting with ASF?

From: Williams, Nekisha (NIH/NIAID) [C]
Sent: Tuesday, May 15, 2018 10:40 AM
To: Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Subject: FW: Meeting Request - Serum Institute Leadership

Good morning Meaghan,

Um following up with you on weather Dr. Fauci is able to do the pre-brief on May 31st or anytime that week?

Thanks,

Nekisha

From: Williams, Nekisha (NIH/NIAID) [C]
Sent: Friday, May 11, 2018 9:51 AM
To: Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Subject: RE: Meeting Request - Serum Institute Leadership

Hi Meagan,

Gray totally forgot he will be traveling to (b)(6) on June 4th would it be possible to do the pre brief on May 31st a time that works for Dr. Fauci?

Thanks,

Nekisha

From: Vance, Meaghan (NIH/NIAID) [C]
Sent: Friday, May 11, 2018 9:22 AM
To: Williams, Nekisha (NIH/NIAID) [C] (b)(6)
Subject: FW: Meeting Request - Serum Institute Leadership

Nekisha:

We can be flexible on June 4th… any time between 8-10am and 1-4pm

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From: Handley, Gray (NIH/NIAID) [E]
Sent: Friday, May 11, 2018 9:09 AM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Cc: Williams, Nekisha (NIH/NIAID) [C] (b)(6)
Subject: RE: Meeting Request - Serum Institute Leadership

That would be fine or even earlier. If he is fine with phone so am I. G

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Friday, May 11, 2018 9:06 AM
To: Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Cc: Handley, Gray (NIH/NIAID) [E] (b)(6)
Subject: FW: Meeting Request - Serum Institute Leadership

Maybe we can do this on June 4 since the 5th is so busy. He can do by phone if need be I think since it is only 15 minutes.

Patricia L. Conrad
Special Assistant to the Director

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From: Handley, Gray (NIH/NIAID) [E]
Sent: Thursday, May 10, 2018 5:30 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Auchincloss, Hugh (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6)
Subject: RE: Meeting Request - Serum Institute Leadership

Great! We will prepare a briefing memo and I can do a pre-brief if you want. That will take about 10 minutes. Let me know a time. Thanks. G

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Thursday, May 10, 2018 5:19 PM
To: Handley, Gray (NIH/NIAID) [E] (b)(6)
Cc: Auchincloss, Hugh (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6)
Subject: RE: Meeting Request - Serum Institute Leadership

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Gray – ASF said he would do this for 30 mins. Am holding 12 pm – 12:30 pm on his calendar. Please advise as to what if any presentation ASF would need.

Also – will you want a pre-brief and when?

thx

Patricia L. Conrad
Special Assistant to the Director

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From: Handley, Gray (NIH/NIAID) [E]
Sent: Tuesday, May 08, 2018 6:25 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Auchincloss, Hugh (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Subject: Meeting Request - Serum Institute Leadership

Would ASF have any time for a 30-60 minute meeting with Dr. Poonawalla and Mr. Poonawalla (father and son) on June 5 any time after 11 a.m.? The Poonawallas are the founder and CEO of the Serum Institute of India, which makes about 60% of the world's vaccines.

They have asked for a courtesy meeting to discuss their interest in working with NIAID and vaccine trials underway. They also own the Serum Institute – which is entirely privately owned.

ASF has previously met Mr. Poonawalla and they hit it off.

Thanks. Gray

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From: Handley, Gray (NIH/NIAID) [E]
Sent: Wed, 30 May 2018 21:45:00 +0000
To: Vance, Meaghan (NIH/NIAID) [C];Conrad, Patricia (NIH/NIAID) [E]
Cc: Auchincloss, Hugh (NIH/NIAID) [E];Hudgings, Carole (NIH/NIAID) [E];Gupta, Ranjan (NIH/NIAID) [E];Meegan, James (NIH/NIAID) [E]
Subject: Briefing documents for Serum Inst Visit June 5, 2018; Pre-brief, May 31, 2018
Attachments: Visitor Bios -SIIPL June 5 2018.docx, SIIPL Profile and NIH-NIAID Engagements - June 2018.docx, India 5_30_2018.docx, ASF-Briefing Memo India-Poonawalla-SII-DRAFT5 6-5-18fgh.doc
Importance: High

Here are materials for tomorrow's pre-brief and the meeting next week with Serum Institute leadership.

Please print and provide to Dr. Fauci and any other participants.

See you tomorrow.

Gray

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From: Mascola, John (NIH/VRC) [E]
Sent: Wed, 16 May 2018 18:56:52 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Marston, Hilary (NIH/NIAID) [E];Eisinger, Robert (NIH/NIAID) [E];Folkers, Greg (NIH/NIAID) [E];Conrad, Patricia (NIH/NIAID) [E];Kwong, Peter (NIH/VRC) [E]
Subject: HIV Ab-based vaccine design Review
Attachments: Kwong, Mascola HIV Ab Review Immunity 2018.pdf

Tony,

This review helps articulate our current thinking on lineage-based and epitope focused vaccine design. The language currently used in the field, such as reverse vaccinology, germline-targeting etc, can be rather arcane and one goal here was to provide a framework to categorize the concepts behind our approaches.

So all of this is "Antibody-based" vaccine design; i.e. taking advantage of what known bNAbs teach us (Dennis calls this "reverse vaccinology")
Two major subcategories are:

1. Epitope-based design – where we use structural knowledge of an epitope on the virus to design the vaccine immunogen (and don't need antibody lineage information)
2. Lineage-based design – were we use knowledge of a specific antibody lineage and its unmutated ancestor (UCA), to design or select an antigen that will trigger the precursor B-cell of the lineage

John

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HIV-1 Vaccines Based on Antibody Identification, B Cell Ontogeny, and Epitope Structure

Peter D. Kwong[1,*] and John R. Mascola[1,*]
[1]Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA
*Correspondence: pdkwong@nih.gov (P.D.K.), jmascola@nih.gov (J.R.M.)
https://doi.org/10.1016/j.immuni.2018.04.029

HIV-1 vaccine development has been stymied by an inability to induce broadly reactive neutralizing antibodies to the envelope (Env) trimer, the sole viral antigen on the virion surface. Antibodies isolated from HIV-1-infected donors, however, have been shown to recognize all major exposed regions of the prefusion-closed Env trimer, and an emerging understanding of the immunological and structural characteristics of these antibodies and the epitopes they recognize is enabling new approaches to vaccine design. Antibody lineage-based design creates immunogens that activate the naive ancestor-B cell of a target antibody lineage and that mature intermediate-B cells toward effective neutralization, with proof of principle achieved with select HIV-1-neutralizing antibody lineages in human-gene knock-in mouse models. Epitope-based vaccine design involves the engineering of sites of Env vulnerability as defined by the recognition of broadly neutralizing antibodies, with cross-reactive neutralizing antibodies elicited in animal models. Both epitope-based and antibody lineage-based HIV-1 vaccine approaches are being readied for human clinical trials.

An inability to elicit—by vaccination—antibodies capable of neutralizing the diverse circulating strains of the human immunodeficiency virus type 1 (HIV-1) that typify transmission has characterized HIV-1 vaccine research for more than 30 years (reviewed in Burton and Mascola, 2015; Escolano et al., 2017; Mascola and Haynes, 2013; Mascola and Montefiori, 2010; Overbaugh and Morris, 2012; Stephenson et al., 2016). This inability stems from features of the virus: its mechanisms of immune evasion and its interaction with the humoral immune system. HIV-1 utilizes an error-prone reverse transcriptase to incorporate its RNA genome into host cells: each time the virus infects a new cell, mutated nucleotides are iteratively incorporated into the diversifying viral genome (Korber et al., 2000; Zhuang et al., 2002). The resultant genetic diversity is amplified during the chronic phase of HIV-1 infection, as evolving virus and developing immune response face off in a struggle that extends in each infected individual over several years (Burton et al., 2005; Mascola and Haynes, 2013). During this time, the brunt of the humoral immune response is directed toward the envelope (Env) spike, the sole focus of virus-directed neutralizing antibodies, with high-titer antibody responses targeting the viral Env glycoproteins – the surface gp120 and the transmembrane gp41, which together comprise the trimeric spike. Viral immune-evasion mechanisms of glycan shielding (Wei et al., 2003) and conformational masking (Kwong et al., 2002) augment evasion by genetic diversity, and HIV-1 stays a few months ahead of the responding immune system (Richman et al., 2003; Wei et al., 2003). The net result is that the viral diversity generated over 6 months in a single HIV-1-infected individual is estimated to equal the diversity generated over a year by the global infection of circulating strains of influenza A virus (Karlsson Hedestam et al., 2008; Korber et al., 2001); with almost 40 million currently infected with HIV-1 (UNAIDS, 2016), the circulating diversity of HIV-1 continues to rise.

Despite extraordinary viral evasion and genetic diversity of HIV-1, semi-conserved regions of Env are vulnerable to neutralizing antibodies. During the months to years of ongoing viral replication, approximately half of those infected by HIV-1 develop cross-reactive serum responses capable of neutralizing ~50% of circulating virus (Hraber et al., 2014). Although HIV-1 evolves to overcome such responses in each of the individuals in which they develop, some individuals develop serum neutralization responses able to neutralize many diverse strains of HIV-1 (Doria-Rose et al., 2010; Gray et al., 2011; Sather et al., 2009; Simek et al., 2009; Stamatatos et al., 2009). It is from the B cells of these individuals that broadly reactive monoclonal antibodies (mAbs) have been isolated. Numerous studies have shown that passive infusion of these neutralizing mAbs, as IgG1 proteins, can protect test animals from viral infection (reviewed in Pegu et al., 2017) and, if infused during infection, can reduce circulating plasma viremia (Barouch et al., 2013; Shingai et al., 2013; and reviewed in Halper-Stromberg and Nussenzweig, 2016). This latter finding has been verified in studies of HIV-1-infected subjects (Caskey et al., 2015, 2017; Lynch et al., 2015; Scheid et al., 2016), and clinical studies are evaluating the ability of antibody VRC01, targeting the CD4-binding site of Env, to reduce the risk of HIV-1 infection (Gilbert et al., 2017; Mayer et al., 2017).

In 2012, we reviewed the identification of broadly neutralizing antibodies, described their molecular recognition including the epitopes on the HIV-1 spike that they target and the molecular trickery they use to achieve their remarkable breadth, and detailed the B cell ontogenies of their generation and maturation (Kwong and Mascola, 2012). Six years later, with the identification and characterization of hundreds of additional antibodies, including VRC-PG05 (Zhou et al., 2018), which targets glycan N262 at the center of the silent face on gp120, the structural basis of antibody recognition targeting all major exposed regions of the prefusion-closed conformation of the HIV-1-Env trimer has now been defined. Moreover, longitudinal sampling with

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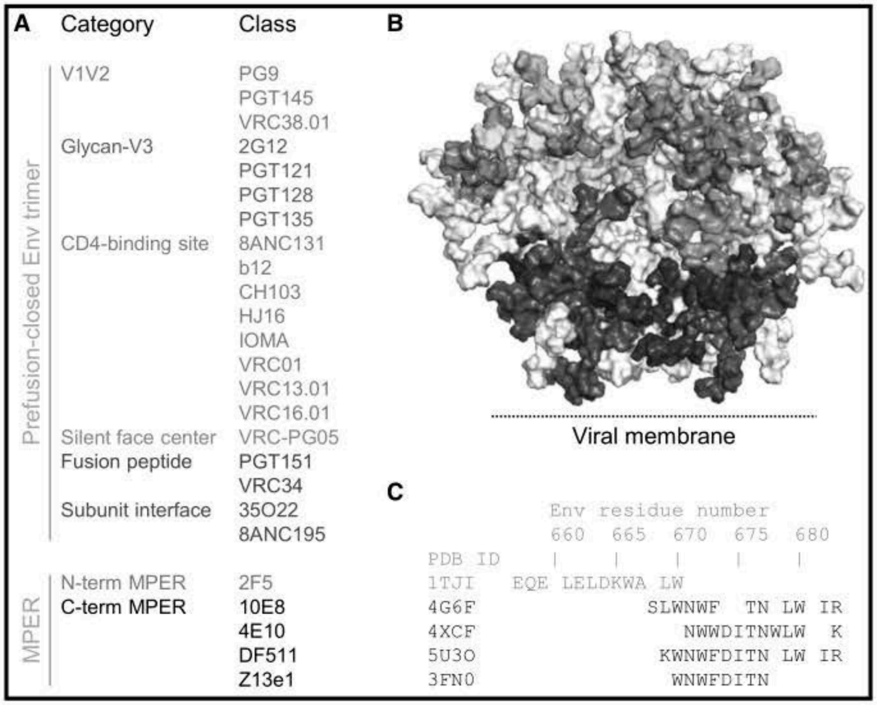

Figure 1. Recognition by HIV-1 Neutralizing Antibodies of the Env Trimer and MPER

(A) Categories and classes of antibodies that neutralize at least 25% of HIV-1 when tested on a cross-clade panel of at least 100 strains. Categories define an epitope or epitope cluster on the viral spike, with the top six categories recognizing the prefusion-closed Env trimer and the bottom two categories recognizing the MPER. Antibody classes are defined by structural mode of recognition and B cell ontogeny and listed by name of representative monoclonal antibody for which a structure has been determined in complex with HIV-1 Env.

(B) The molecular surface of the prefusion-closed Env trimer is shown with epitopes colored by category. Residues belonging to multiple categories were colored according to the category for which recognition was most critical; for example, glycan N276 is accommodated by CD4-binding site antibodies, but required for 8ANC195 binding, so N276 has been colored as part of the subunit interface category. Overall, the prefusion-closed trimer is recognized by 20 structurally defined classes of neutralizing antibodies that can be delineated by epitope into six antibody categories (V1V2 through subunit interface). Figure adapted from Chuang et al. (2018).

(C) The membrane-proximal external region (MPER) of the viral spike is recognized by five structurally defined classes of neutralizing antibody that can be delineated by epitope into two categories. Residues that comprise each epitope are listed along with source PDB ID and colored as in (A) by category.

next-generation sequencing (NGS) of B cell transcripts has transformed our understanding of B cell lineage development; i.e., how neutralizing antibody lineages arise and evolve via somatic hypermutation (SHM) to achieve cross-reactive neutralization. Here we review molecular mechanisms of immune recognition for broadly neutralizing antibodies, detail pathways of antibody development, and describe how structural and immunological information is informing vaccine design. Specifically, we describe antibody-to-vaccine strategies of vaccine design, focusing on antibody lineage-based design and on epitope-based vaccine design, both of which have recently achieved substantial progress toward this goal.

Comprehensive Recognition by Broadly Neutralizing Antibody of the Spike Ectodomain

Over the last 10 years, B cell culture and antigen-specific probe-based sorting of B cells from HIV-infected donors have combined with single-cell sequencing of immunoglobulin transcripts to transform the identification of broadly neutralizing antibodies (Scheid et al., 2011; Walker et al., 2009; Wu et al., 2010). This transformation in identification has been paralleled by a transformation in our ability to determine antibody-Env structures especially with near-native Env trimers (Gristick et al., 2016; Julien et al., 2013a; Lee et al., 2016; Lyumkis et al., 2013; Pancera et al., 2014; Stewart-Jones et al., 2016). Antibodies capable of neutralizing at least 25% of HIV-1 strains have now been identified that recognize all major exposed surfaces of the prefusion-closed Env trimer (Env residues 32–664) (Figures 1A and 1B) as well as all sequence segments of the highly conserved membrane-proximal external region (MPER) of the spike ectodomain (Env residues 660–683) (Figure 1C). Although Env trimer-directed antibodies and MPER-directed antibodies can be coaxed into recognizing the same Env construct, such as with the detergent-solubilized Env trimer recognized by PGT151 and 10E8 antibodies (Lee et al., 2016), Env trimer-directed and MPER-directed antibodies appear to target different conformations of the spike ectodomain: the former recognize the prefusion-closed conformation of the Env trimer prior to engagement of CD4 (Chuang et al., 2018; Munro et al., 2014), whereas the latter appear to recognize a conformational of the spike ectodomain further along the Env-entry pathway (Chakrabarti et al., 2011; Ruprecht et al., 2011).

On the prefusion-closed trimer (Figure 1B), viral immune-evasion mechanisms of glycan shielding and sequence diversity dominate. The spike apex in particular is covered by multiple glycans and variable loops of high sequence variability. V1V2-directed broadly neutralizing antibodies such as PG9 and PGT145 use anionic protruding loops to reach past the glycan shield to interact with locally conserved pockets of cationic conservation (Lee et al., 2017; Liu et al., 2017; McLellan et al., 2011b), while antibody VRC38.01 (Cale et al., 2017) recognizes a similar site of vulnerability but utilizes side chain-to-side chain recognition to reduce the length of the loop needed to transverse the glycan shield. Glycan-V3-directed broadly neutralizing antibodies such as PGT121, PGT128, and PGT135 also utilize protruding loops to reach pockets of conservation (Julien et al., 2013b; Kong et al., 2013; Pejchal et al., 2011), often associated with function such as coreceptor recognition (Sok et al., 2016b). These antibodies recognize glycan directly, especially a high mannose patch which encompasses glycan N332. For 2G12, recognition involves a glycan cluster without protein

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contacts (Sanders et al., 2002; Scanlan et al., 2002), which it achieves using domain swapping to increase the size of the paratope (Calarese et al., 2003).

Further toward the spike stem region, antibodies that target the CD4-binding site predominate. While mechanisms of recognition focused on the 3rd-heavy chain complementarity determining region (CDR H3) occur with antibodies b12, CH103, HJ16, VRC13, and VRC16 (Liao et al., 2013; Zhou et al., 2007, 2015), V-gene mechanisms of recognition involving VH1-2 or VH1-46 germline origin genes are also observed with antibody classes including that of VRC01, 8ANC131, and IOMA (Gristick et al., 2016; Zhou et al., 2010, 2015). CD4-binding site antibodies often require extraordinary levels of SHM to achieve neutralization potency and breadth (Zhou et al., 2015), a process assisted by the intrinsic mutability of antibody V-genes (Bonsignori et al., 2016).

At the center of the silent face, glycan N262 has withstood the identification of neutralizing antibodies. Antibody VRC-PG05, however, was recently observed to utilize a double-protruding loop mechanism to recognize glycan N262 along with glycans N295 and N448 (Zhou et al., 2018). This antibody—the sole current member of the "silent face center" category—recognizes an epitope comprising 88% glycan, neutralizes 27% of HIV-1 strains, and is easily escaped by alteration of glycan N448 or glycan-proximal residue E293.

Further toward the membrane, two antibodies—PGT151 (Lee et al., 2016) and VRC34.01 (Kong et al., 2016)—recognize glycopeptide epitopes that encompass the N terminus of the fusion peptide, the component of the HIV entry machinery which anchors the Env-prehairpin intermediate in the target cell membrane and which requires proteolytic activation (reviewed in Colman and Lawrence, 2003). These antibodies recognize elements of both gp120 and gp41 and along with antibody ACS202 (van Gils et al., 2016) comprise the "fusion peptide" category of broadly neutralizing antibodies. Despite the fusion peptide being the characteristic target of this category of antibody, each antibody appears to recognize the fusion peptide differently. Antibodies 35O22 (Huang et al., 2014) and 8ANC195 (Scharf et al., 2014) comprise the "subunit interface" category of antibodies recognizing the prefusion-closed conformation of Env. These antibodies are distinguished by their recognition of protein and glycans emanating from both gp120 and gp41 subunits (although 8ANC195 can bind gp120 in the absence of gp41) and by their tolerance of gp120 conformational change (Scharf et al., 2015). These antibodies have breadths of ~50% and may have high potency (35O22).

Lastly, with the glycan-free sequence-conserved MPER (Figure 1C), viral immune-evasion mechanisms of glycan shielding and high sequence diversity take a back seat to mechanisms of conformational masking, steric occlusion, and membrane proximity. Antibody 2F5 recognizes an extended conformation of the N-terminal half of the MPER (Ofek et al., 2004), whereas antibodies 10E8, 4E10, DH511, and Z13e1 recognize a characteristic helix at the C-terminal half of the MPER (Cardoso et al., 2005; Huang et al., 2012; Pejchal et al., 2009; Williams et al., 2017a).

Overall, the identification of broadly neutralizing antibodies that recognize all major exposed regions of the prefusion-closed trimer as well as all residues of the MPER demonstrates the ability of the human immune system to bypass glycan shielding, sequence variation, and conformational masking to recognize comprehensively the spike ectodomain. In addition to expanding our understanding of human antibodies, these antibodies (and their variants engineered for greater potency, breadth, and half-life) may impact both treatment and prevention of HIV-1 through near pan-reactive antibodies such as VRC07-523LS or N6 (95%–97% breadth) (Huang et al., 2016; Rudicell et al., 2014), which targets the CD4-binding site, or 10E8v4-5R+100cF (98% breadth) (Kwon et al., 2018), which targets the C-terminal region of the MPER.

Developmental Pathways of Broadly Neutralizing Antibodies by Longitudinal Sampling

Although substantial insight into B cell ontogeny can be gained from analysis of mature antibodies, longitudinal assessment of the memory B cell compartment coupled to next-generation sequencing of immunoglobulin transcripts provides an in-depth means to address questions about initial V(D)J recombination, CDR H3 length, and SHM. Indeed, over the last 5 years, longitudinal analyses of developing B cell lineages—several sampled from close to time of infection—have been reported. To date, detailed B cell genetic studies of longitudinal samples have defined the developmental pathway of only a few neutralizing antibody lineages, targeting the CD4-binding site, the V1V2, and the glycan-V3 (Bonsignori et al., 2016; Doria-Rose et al., 2014; Landais et al., 2017; Liao et al., 2013; MacLeod et al., 2016; Simonich et al., 2016).

In 2013, Liao et al. (2013) reported analysis of a donor enrolled in the CHAVI001 acute HIV-1 infection cohort approximately 4 weeks after HIV-1 infection (Figure 2, top panels). Consistently high viral loads were observed over years of infection, often above 10^5 plasma RNA copies per milliliter. Antibody CH103 identified from week 136 neutralized 55% of HIV-1 strains through recognition of the CD4-binding site by a CDR H3-based mechanism. Analysis of evolving virus and developing CH103 lineage revealed lineage transcripts as early as 14 weeks after infection. Early CH103-lineage members showed modest neutralization, with the lineage requiring more than 100 weeks of development to achieve 50% breadth. Analysis of co-evolving virus and CH103 lineage indicated co-evolution with another antibody lineage, and probe-sorting identified a second CD4-binding site lineage, named CH235, from week 41 (Gao et al., 2014), with modest breadth (19%) and potency. Tracking lineage development though NGS of immunoglobulin transcripts indicated this second lineage slowly gained breadth such that CH235.12 isolated from week 323 after infection had 90% breadth, with an average potency of 0.6 μg/mL (Bonsignori et al., 2016). Notably, just two lineages appeared to account for much of the neutralization breadth from donor CH505. Analysis of other donors, such as donor NIAID-45, the source of antibody VRC01, also indicate an extensively diversified B cell lineage (the VRC01 lineage) to account for much of the neutralization from this donor (Wu et al., 2015). Overall, longitudinal sampling revealed lineage development of CD4-binding site antibodies to extend over years, often with extensive SHM to develop breadth and potency.

In 2014, Doria-Rose et al. (2014) reported analysis of a donor enrolled in the CAPRISA cohort approximately 6 weeks after

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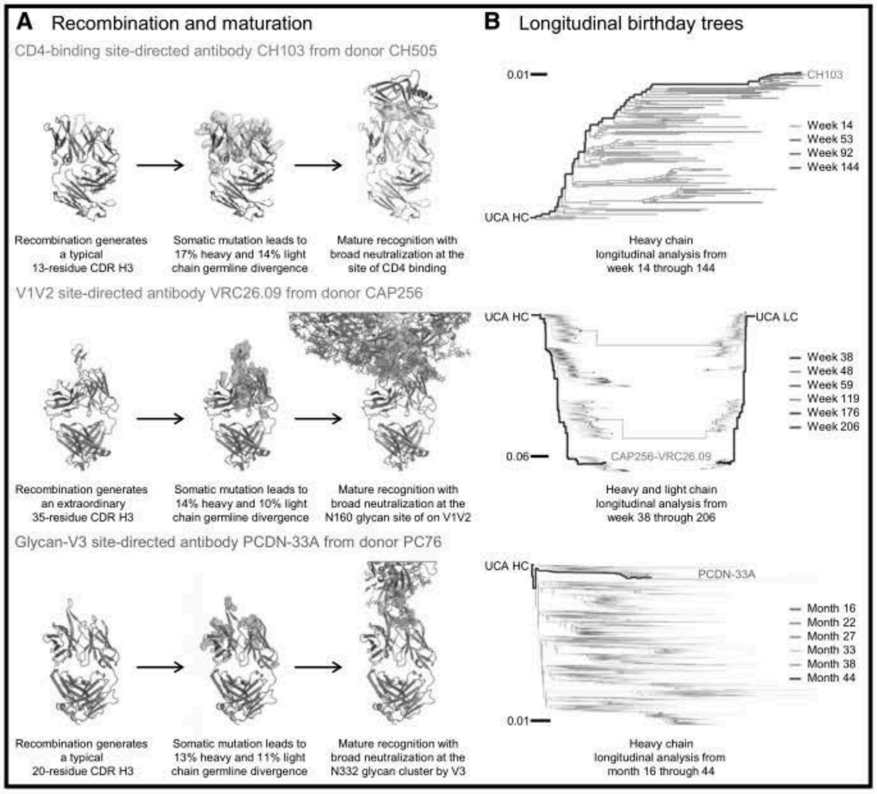

Figure 2. B Cell Ontogenies by Longitudinal Sampling and NGS

Antibodies capable of neutralizing diverse strains of HIV-1 generally have unusual features, a result of unusual recombination, extensive somatic hypermutation (SHM), or both.

(A) Recombination and maturation. CD4 binding site-directed antibodies may have extraordinary levels of SHM of more than 100 nucleotide changes, such as for VRC01-class antibodies (Wu et al., 2011). Shown is the CH103 antibody (Liao et al., 2013), which has a somewhat atypical SHM level, roughly 2 standard deviations above the mean human level of 8.7 ± 4.1% nucleotide SHM changes (top panel). V1V2-directed antibodies have less SHM, but CDR H3s loops of extraordinary length, such as shown for antibody CAP256-VRC26.09, which has a CDR H3 length more than 6 standard deviations above the mean (13.9 ± 3.5 residues) (middle panel). Glycan-V3 antibodies often have typical to somewhat atypical CDR H3 lengths and SHM levels (bottom panels).

(B) Longitudinal birthday trees. Next-generation sequencing of B cell transcripts, when coupled to longitudinal sampling of the B cell repertoire, allows for a time-sensitive phylogenetic characterization of the development of select B cell lineage. Shown are phylogenetic trees from the CH103 lineage (Liao et al., 2013) (top panel), from the CAP256-VRC26 lineage (Doria-Rose et al., 2014), and from the PCDN-33A lineage (MacLeod et al., 2016).

HIV-1 infection (Figure 2, middle panels). B cell culture of longitudinal samples from weeks 59, 119, and 206 from time of infection identified 12 mAbs from the CAP256-VRC26 lineage, which uses an unusually long (35 residues) tyrosine sulfated-CDR H3 to recognize a V1V2 epitope. Assessment by NGS of B cell transcripts indicated the unmutated ancestor of the lineage to have emerged between weeks 30 and 38 after infection. Improved neutralization breadth occurred by week 59 with modest affinity maturation. HIV-1 V1V2-directed neutralizing antibodies could thus develop relatively rapidly through initial selection of B cells with a long CDR H3 and limited subsequent SHM. Similarly, an antibody lineage with a 25-residue CDR H3 and relatively rapid development of breadth (in about 14 months) with only 10% SHM was observed by Landais et al. (2017) in a donor from IAVI's African HIV Research Network. These V1V2 results—both involving antibodies of the PG9 class—showed substantial contrast to the CD4-binding site lineages, CH103, CH235, and VRC01, all of which required higher SHM, suggesting that antibody lineages targeting different sites of vulnerability have different developmental characteristics.

In 2016, MacLeod et al. (2016) reported analysis of donor PC76 from the IAVI Protocol C, with serum and B cells analyzed longitudinally from 5 to 38 months after infection (Figure 2, bottom panels). Antibodies targeting the glycan-V3 site of vulnerability were observed to develop in a lineage (PCDN), which arose sometime between month 10 and 16 after infection and achieved substantial neutralization breadth by month 38 with ~15% SHM. Next-generation sequence analysis of a B cell transcripts revealed a complex lineage involving multiple independently sublineages that diversified early, before developing into divergent antibodies with diverse recognition of the target site.

Overall, NGS with longitudinal sampling has provided unparalleled insight into the development of broadly neutralizing antibodies (Bonsignori et al., 2016, 2017; Doria-Rose et al., 2014; Landais et al., 2017; Liao et al., 2013; MacLeod et al., 2016; Wu et al., 2011, 2015). Development times and SHM levels required to achieve neutralization breadth appear to depend on characteristics related to each specific site of vulnerability and to range from multiple years to as little as 6 months. Broadly neutralizing lineages appear to be rare, with just a handful in each donor. However, extensive maturation allows even single lineages to expand and to dominate the neutralizing response.

Antibody Lineage-Based Vaccine Design

The detailed immunological studies described above provide evidence that the expansion of a single antibody lineage can, in some cases, account for a substantial proportion of total serum neutralization potency and breadth. This is highlighted by the strong correlation between neutralization of serum and the neutralization of select isolated mAbs (Bonsignori et al., 2012; Georgiev et al., 2013; Simonich et al., 2016; Walker et al., 2010; Wu et al., 2010) and suggests that the elicitation of a specific antibody lineage could be a key goal of HIV-1 vaccine design (Bonsignori et al., 2017; Haynes et al., 2012; Kwong and Mascola, 2012; Mascola and Haynes, 2013; Stamatatos et al., 2017). Antibody lineage-based vaccine design (also called B cell ontogeny-based vaccine design) is predicated on reproducing or "re-eliciting" a target antibody class and benefits from definition of the earliest stage of a neutralizing antibody lineage

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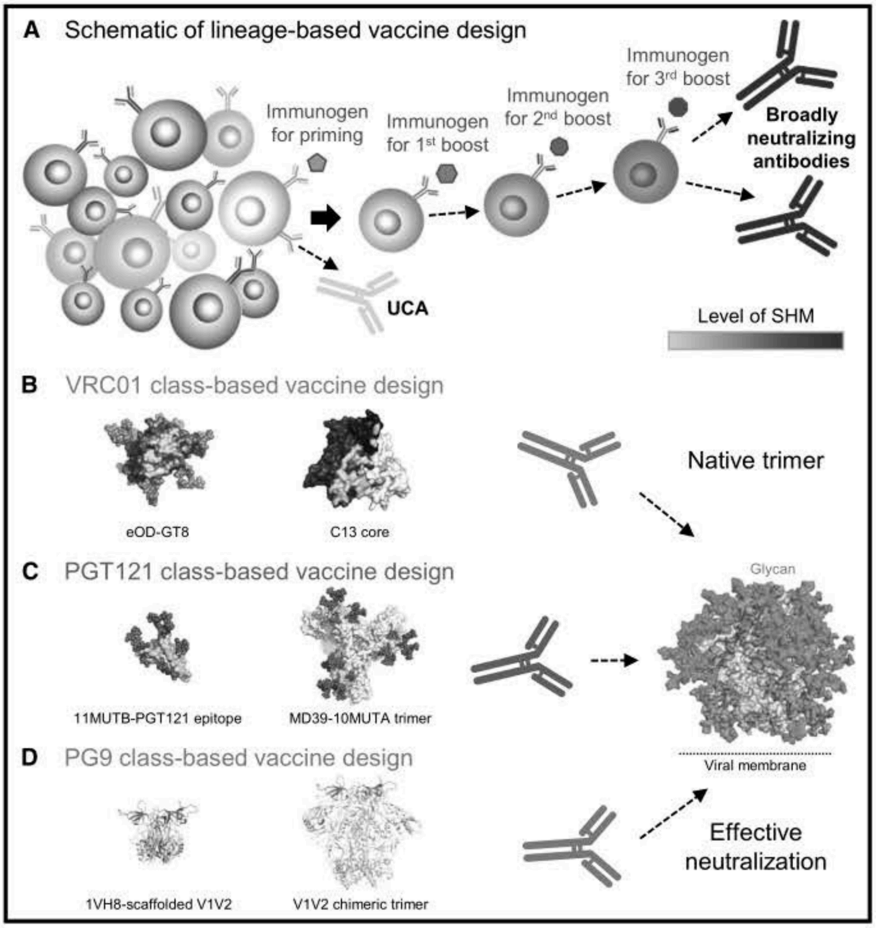

Figure 3. Antibody Lineage-Based Vaccine Design

(A) An antibody lineage is initiated from a single precursor B cell along with its secreted antibody, termed the unmutated common ancestor (UCA) of the lineage. Lineage-based vaccine design uses knowledge of the inferred UCA to design an appropriate UCA-priming antigen. Once triggered, the lineage can be further boosted by modified Env-based immunogens designed to engage lineage intermediates and to promote affinity maturation toward effective neutralization. (B–D) Vaccine antigens have been designed to trigger and to mature antibody lineages for (B) VRC01-class antibodies (Jardine et al., 2015; Tian et al., 2016), (C) PGT121-class antibodies (Steichen et al., 2016), and (D) PG9-class antibodies (Gorman et al., 2016).

(Figure 3A). From the B cell perspective, this is the B cell receptor (BCR) of the naive ancestor-B cell from which the entire lineage was derived. From the perspective of secreted antibody, this is the unmutated common ancestor (UCA) of the lineage. Since mature broadly neutralizing antibodies arise though substantial affinity maturation, it can be difficult to infer the UCA which arises from gene recombination and non-templated nucleotide additions (Schatz and Spanopoulou, 2005). It is often possible to infer the most likely germline V and J genes for the heavy and light chain, but the recombination leading to the heavy and light chain CDR-3 regions is more difficult. Inference of the UCA is most robust when there are numerous members of an antibody family including less mutated members. This can be achieved by using NGS of specific antibody gene transcripts and then inferring the UCA based on phylogenetic or statistical tools (Doria-Rose et al., 2014; Kwong et al., 2017; Liao et al., 2013; MacLeod et al., 2016). Thus, the accuracy of the inferred UCA may vary, depending on the number of antibody sequences and on availability of samples from early time points in the evolution of the antibody lineage.

The field of lineage-based design has been influenced by observations that germline-reverted versions of mature broadly neutralizing antibodies (i.e., V-region, and sometimes J-region, sequences reverted to inferred germline-gene sequence) often lack measurable binding to most gp120 or related Env glycoproteins (Andrabi et al., 2015; Gorman et al., 2016; Hoot et al., 2013; Jardine et al., 2013; McGuire et al., 2013; Xiao et al., 2009; Zhou et al., 2010). The ensuing hypothesis for lineage-based vaccine design is that induction of a specific antibody class may require targeting with an immunogen that binds the UCA with sufficient affinity to activate the appropriate BCR (Bonsignori et al., 2017; Escolano et al., 2017; Haynes et al., 2012; Jardine et al., 2013; Mascola and Haynes, 2013; Stamatatos et al., 2017). Once triggered, the lineage could be further boosted by modified Env-based proteins designed to engage lineage intermediates and to promote affinity maturation toward effective neutralization. Thus, germline-reverted versions of known broadly neutralizing antibodies have been used to characterize Env antigens and, based on screening of Env variants and structural understanding of the epitope, to design antigens with improved binding avidity to the UCA. This approach has had most success with "public" antibody classes, which are observed in multiple donors, such as the VRC01 class (Zhou et al., 2013). The term "germline-targeting" immunogens is often used to describe this effort, but since the antigen is designed to target a naive ancestor B cell expressing a recombined BCR which is often the product of exonuclease trimming and non-template nucleotide additions (Schatz and Spanopoulou, 2005), the term germline here is used liberally—referring to the inferred germline V(D)J genes that make up the precursor BCR.

Lineage-based design has also been informed by longitudinal studies that define the sequence of the initial viral strain causing infection—often termed the transmitted-founder (TF) virus (Derdeyn et al., 2004; Keele et al., 2008). Studies such as those described above that define both the TF virus and the UCA of a dominant neutralizing antibody lineage show that while the UCA of a broadly neutralizing antibody lineage may not bind to most Env proteins, it can in some cases recognize the TF Env (Doria-Rose et al., 2014; Liao et al., 2013). Thus, longitudinal seroconverter cohorts have proved to be invaluable by providing

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key information on both the antibody lineage and the TF viral sequence that can be used for antigen design. The first example of lineage-based vaccine design to enter phase I human testing comes from the CD4-binding site CH103 lineage described above (Liao et al., 2013) with supporting data in animal models (Williams et al., 2017b). Here, the priming gp120 antigen is based on the TF Env isolated from the donor, and two sequential boost gp120 were chosen from later time points, as the Env evolved in response to the antibody lineage. Thus, booster Envs were selected due to loss of reactivity with early stage, less mutated, lineage antibodies, but that maintained binding affinity for the mature CH103 antibody. In this way, booster Envs are chosen to promote affinity maturation toward the pathway of neutralization breadth (Bonsignori et al., 2017). In summary, lineage-based vaccine design uses knowledge of inferred ancestors of a neutralizing antibody lineage to design or to select optimized immunogens (Figure 3). Since we define a class of antibodies as those sharing genetic characteristics and structural mode of recognition, the concept of lineage-based design can be applied to all members of each antibody class.

VRC01 Class Lineage-Based Design

A major target for lineage-based design is the VRC01 class of antibodies, which target the relatively conserved CD4-binding site and, through affinity mutation, acquire potency and high breadth of reactivity (Figure 3B; Scheid et al., 2011; Wu et al., 2010, 2011; Zhou et al., 2010). VRC01-class antibodies, including VRC01, 12A21, 3BNC117, and N6, share common genetic features such as the same heavy chain gene, VH1-2*02, which makes key Env contacts via its CDR H2, and a 5 amino acid CDR L3 required to avoid clashes with the V5 region of Env (Scheid et al., 2011; West et al., 2012; Wu et al., 2011; Zhou et al., 2010, 2013, 2015). An important feature of the VRC01 class is that it has been found in numerous HIV-1-infected donors and thus represents a reproducible class of neutralizing antibodies (Zhou et al., 2013, 2015). While a formal UCA of VRC01-class antibodies has not been defined, initial studies demonstrate that germline-reverted variants of VRC01 display little or no reactivity to gp120 (Hoot et al., 2013; Jardine et al., 2013; McGuire et al., 2013; Scheid et al., 2011; Zhou et al., 2010, 2013). This was a surprising observation because mature VRC01 binds with high affinity to most strains of gp120. Based on insights from antigen screening and structural mode of VRC01-class antibody recognition, investigators have created gp120 variants that lack several key glycans, including glycan N276. Mature VRC01 accommodates and interacts with N276, but the steric shielding from N276 and other glycans surrounding the CD4-binding site makes it difficult for less mature and lower affinity versions of VRC01 to navigate the appropriate angle of approach and to recognize the CD4-binding site (Zhou et al., 2015). Thus, several versions of gp120 molecules have been engineered that interact with germline-reverted VRC01-class antibodies. Examples include the engineered outer domain of gp120 (eOD-GT6, eOD-GT8) (Jardine et al., 2013, 2016a), engineered C13 core gp120 (Tian et al., 2016), engineered variants of 426c gp140 (McGuire et al., 2013, 2016), and most recently, near-native trimers (Medina-Ramírez et al., 2017). Many of these antigens have been tested in mouse models where the rearranged heavy chain, and sometimes also the light chain, of VRC01 class antibodies were integrated into the mouse IgG loci. In such "knock-in" mouse models, differing levels of B cells can be induced to express the VRC01 class precursor antibody—allowing for a range of immunological assessment of test immunogens.

Initial knock-in mice studies have provided proof of principle for HIV-1 lineage-based design (reviewed in Crowe, 2016; Verkoczy et al., 2017). Immunization of mice expressing the germline-reverted rearranged VRC01 or 3BNC60 heavy chain resulted in expansion of B cells in which the human VRC01-class heavy chain was paired with the requisite 5 amino acid CDR L3 region of the light chain derived from the mouse genome (Briney et al., 2016; Dosenovic et al., 2015; Jardine et al., 2015; McGuire et al., 2016; Tian et al., 2016). Thus, early stage VRC01 class-antibody lineages could be expanded, though these antibodies lacked enough affinity maturation to neutralize wild-type strains of HIV-1. Additional evidence has derived from immunization of mice expressing the human VH1-2*02 allele that undergoes normal V(D)J recombination with endogenous mouse D and J gene segments to form precursor VRC01 class antibodies with diverse CDR H3 regions (Tian et al., 2016). In total, these studies have provided important proof of principle; i.e., it was possible to engineer an immunogen to target a specific BCR and expand an antibody lineage. These studies also examined sequential boosting regimens. For example, priming immunogens without glycan N276 were used to promote improved affinity for VRC01 class BCRs, and this glycan was added back to subsequent boosting immunogens (Briney et al., 2016; Tian et al., 2016). While sequential boosts could promote affinity maturation of VRC01 class antibodies, mAbs isolated from these regimens preferentially neutralized Env pseudoviruses that lacked the N276 glycan. Thus, despite the general over-representation of B cells with human VRC01 class characteristics in these mouse models, current prime and boost immunogens have not yet induced enough affinity maturation to generate robust VRC01-class neutralization activity.

Several groups of investigators continue to work on strategies to improve antigen design and to define strategies of sequential boosting with Envs designed to boost intermediate VRC01 class precursor antibodies—with the goal of inducing enough affinity maturation to allow these antibody lineages to engage the CD4-binding site in the context of the native trimer with native glycosylation, thus inducing virus neutralization. Evidence that optimal regimens may benefit from a native trimer boost comes from a study of transgenic mice expressing the mature recombined heavy chain of 3BNC60. Here, neutralizing antibodies were only generated when the germline targeting immunogen was boosted with a native-like trimeric protein (Dosenovic et al., 2015). The design of improved immunogens, and immunization regimens, will continue to be informed by knock-in mouse studies, but proof of concept for lineage-based vaccine design will require human studies. Encouraging evidence comes from both studies of mice transgenic for human immunoglobulin loci (Sok et al., 2016a) and *ex vivo* studies of human B cells, showing that the "germline-targeting" immunogen eOD-GT8 could be used as a protein probe to isolate VRC01-class naive B cells from HIV uninfected donors (Jardine et al., 2016a). Currently, there are plans to develop clinical grade eOD-GT8 and 426c-based germline binding Env immunogens—in both cases, likely

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taking advantage of self-assembling nanoparticle display formats, such as ferritin or lumazine synthase, to improve immunogenicity (Georgiev et al., 2018; Jardine et al., 2013; Kanekiyo et al., 2013; Zhao et al., 2014). In addition, several forms of native-like trimer proteins have been designed and some are currently in development for clinical testing (Chuang et al., 2017; de Taeye et al., 2015; Georgiev et al., 2015; Guenaga et al., 2017; Joyce et al., 2017; Kwon et al., 2015; Medina-Ramírez et al., 2017; Rutten et al., 2018; Sharma et al., 2015; Torrents de la Peña et al., 2017; reviewed in Sanders and Moore, 2017). The development of these immunogens will pave the way for human studies to test the concept of lineage-based design for VRC01-class antibodies.

PGT121 Class Lineage-Based Design

Neutralizing antibodies targeting glycan-V3 region of Env are commonly detected in broadly reactive neutralizing sera. In one large longitudinal study of HIV-1 infection in African countries, nearly half of those developing broad serum neutralization had evidence of antibodies targeting the glycan-V3 epitope (Landais et al., 2016), and several classes of such antibodies have been defined by isolated mAbs (Burton and Hangartner, 2016; Sok et al., 2013). This makes the glycan-V3 category an attractive target for vaccine design. Among this category of broadly neutralizing antibodies, the PGT121 lineage has been particularly well characterized, including binding and neutralization properties and structural mode of recognition (Garces et al., 2014, 2015; Julien et al., 2013a, 2013b; Mouquet et al., 2012; Pancera et al., 2014; Sok et al., 2013, 2014a; Walker et al., 2011). Moreover, the identification of an antibody with similar CDR H3 recognition as PGT121—antibody BG18 (Barnes et al., 2018)—shows the PGT121-based mode of recognition to be reproducible.

PGT121-class antibodies recognize the N332 glycan, along with several proximate glycans and V3 region amino acids. While UCAs for PGT121-class antibodies have not been accurately delineated, the numerous members of the PGT121 lineage studied have allowed for robust inference of germline genes and, thus, a germline-reverted version of PGT121. As for VRC01-class antibodies, the predicted germline-reverted version of PGT121 lacks detectable binding to native forms of Env (Sok et al., 2013), providing motivation for lineage-based vaccine design. Steichen et al. (2016) approached this design using a structure-guided, directed evolution approach and mammalian cell-surface display to identify variants of Env trimers with affinity for germline-reverted precursors of PGT121 (Figure 3C); they tested these immunogens in knock-in mice expressing the PGT121 rearranged inferred germline heavy chain (or an intermediate version of the heavy chain), together with the rearranged inferred germline light chain. Priming immunogens elicited precursor PGT121-class antibodies, and boosting immunogens were chosen based on binding to these precursor antibodies (Escolano et al., 2016). Thus, a set of sequential immunogens, followed by native-like trimer, generated PGT121-class antibodies capable of neutralizing diverse strains of HIV-1. While promising, we note that re-eliciting glycan-V3 lineages, which depend on CDR H3 modes of recognition, may potentially be more difficult than re-eliciting modes of recognition that depend on conserved V-gene features (such as with the VRC01 class). Nevertheless, the development of glycan-V3 lineages over time, together with virus co-evolution, have recently been elucidated from three donors (Bonsignori et al., 2017; MacLeod et al., 2016; Simonich et al., 2016), thus providing detailed genetic information to facilitate lineage-based design. In addition, the identification of glycan-V3 antibodies that lack affinity maturation-derived insertions or deletions may provide more attractive targets for lineage-based vaccine design (Freund et al., 2017).

PG9 Class Lineage-Based Design

There are also data to support lineage-based design of V1V2 mAbs (Figure 3D) that often share particular genetic features and binding characteristics, including long anionic CDR H3 regions to engage and reach past surface glycans and interact with the underlying protein surface. Based on genetic characteristics and structural mode of recognition, the PG9 class includes CAP256-VRC26 and CH01 lineages (Bonsignori et al., 2011; Doria-Rose et al., 2014; Gorman et al., 2016), while PGT145 forms a separate class (Lee et al., 2017; Liu et al., 2017) which includes its more potent lineage member PGDM1400 (Sok et al., 2014b), and VRC38 also forms its own class (Cale et al., 2017). Detailed antibody lineage and virus co-evolution studies demonstrate that the UCA of the CAP256-VRC26 lineage displayed modest recognition of the donor TF Env, but little recognition of most other Envs (Bhiman et al., 2015; Doria-Rose et al., 2014, 2015). This suggests favorable features of the TF as a potential immunogen, though these characteristics are not yet fully understood. Subsequent studies demonstrate that the UCA of CAP256-VRC26 preferentially shares binding characteristics with germline-reverted versions of antibodies PG9 and CH01; i.e., each of these precursor antibodies recognize a small but similar set of HIV Env trimers (Andrabi et al., 2015; Gorman et al., 2016).

Overall, these data suggest that lineage-based design using Env antigens with enhanced sensitivity to germline reversions of V1V2 antibodies may have the potential to target multiple lineages within the PG9 class of V1V2-neutralizing antibodies (Andrabi et al., 2015; Gorman et al., 2016; Moore et al., 2017). This may be important because the uncommon gene recombination events producing long CDR H3s of these antibodies results in a low potential precursor frequency (Briney et al., 2012b; Jardine et al., 2016b). Voss et al. (2018) further analyze these sensitive Envs and identify a common glycan hole, i.e., strains lacking glycan N130 commonly found in the V1V2 region. This provides a potential mechanism for the sensitivity of these Envs and has furthered interest in using such Envs as immunogens. Immunization of rabbits with several such Envs has generated V1V2-directed neutralizing antibodies, though mainly to the virus that is autologous to the immunizing strain (Voss et al., 2018). As native trimer immunogens progress toward clinical development, it may be prudent to include a trimer such as from strains WITO.33 or ZM233.6 (Gorman et al., 2016) or from strain CRF02_AG_250 (Andrabi et al., 2015) with favorable characteristics for engaging precursor B cells of PG9-class antibodies. As with all lineage-based design, engineering of optimal boosting immunogens may benefit from knowledge of antibody-virus co-evolution, such as described by Bhiman et al. (2015) for the CAP256-VR26 lineage or by Landais et al. (2016) for the PC64

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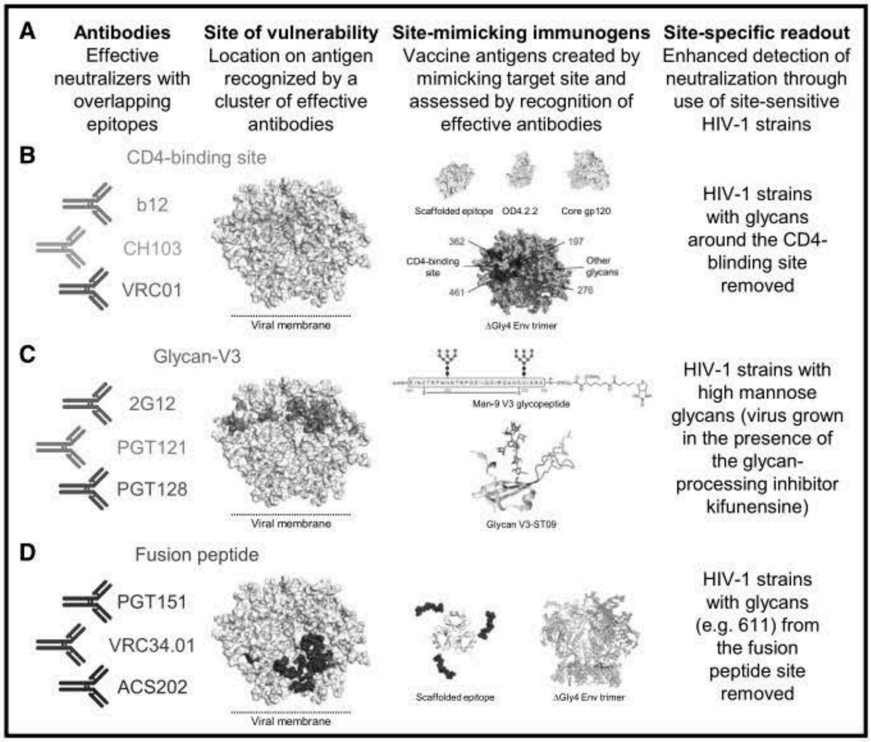

Figure 4. Epitope-Based Vaccine Design

(A) Overall process of epitope-based vaccine design involves identification of antibodies, definition of a target site, and design and immunization using site-mimicking immunogens, followed by immunological measures, such as epitope-specific neutralization, to assess success (site-specific readout). Importantly, multiple antibodies from the same category but from different classes can be used to assess immunogens.

(B–D) Leftmost panels identify multiple template antibodies, second panels from left show epitope-defined supersites on the prefusion-closed Env trimer, second panels from right show various immunogens designed to mimic the target supersite, rightmost panels delineate site-specific neutralization.

(B) CD4-binding site-mimicking immunogens include (top) core gp120 (Zhou et al., 2007), OD4.2.2 (Joyce et al., 2013), and an epitope scaffold (Azoitei et al., 2011); (bottom) BG505 DS-SOSIP.664 with glycans around the CD4-binding site removed (Zhou et al., 2017).

(C) Glycan-V3 site-mimicking immunogens include (top) Man-9 glycopeptide (Alam et al., 2017) and (bottom) ST09-epitope scaffold (Zhou et al., 2014).

(D) Fusion peptide site-mimicking immunogens include (left) fusion peptide epitope scaffold (Kong et al., 2016; Xu et al., 2018) and (right) Env trimer with glycans around the fusion peptide removed.

lineage. Thus, additional antibody-virus co-evolution studies would provide useful information for PG9-class antibody-based lineage design. Finally, there may be advantages to eliciting antibodies that lack highly unusual features, such as long CDR H3 loops, though for V1V2-category neutralizing antibodies, only one such example has been observed (Cale et al., 2017).

Epitope-Based Vaccine Design

In addition to lineage-based vaccine design, information gleaned from antibody identification, from their structures in complex with Env, and from epitope-defined sites of vulnerability provides a basis for epitope-based vaccine design (Figure 4A). Such design involves (1) identification of broadly neutralizing antibodies with overlapping epitopes, (2) delineation of a vulnerable site on Env recognized by the cluster of effective antibodies, (3) design of immunogens that mimic critical elements of the Env site as judged by antigenic reactivity to site-specific antibodies, and (4) immunization in test animals with readout that is selective to target epitope-directed antibodies (Azoitei et al., 2011; Correia et al., 2014; McLellan et al., 2013a; Ofek et al., 2010a; Xu et al., 2018; Zhou et al., 2014, 2017). One critical attribute of epitope-based design that distinguishes it from lineage-based design is that the site-specific antibodies used to assess antigens can derive from different classes of antibodies (whereas lineage-based design is class specific). Thus, different immunological pathways can be used to generate site-specific antibodies, with antigenic assessment identifying epitope features common to the category. Once desired antibodies are elicited, iterative structure-based improvement can be used to improve elicited antibody responses (Joyce et al., 2016b; Xu et al., 2018).

Epitope-based vaccine design has been tried for several HIV-1-neutralizing antibodies. With CD4-induced antibodies like 17b and 48d, which bind to the CD4-induced co-receptor binding region, structure-based stabilization of core gp120 into the CD4-bound conformation induces antibodies that recognize the co-receptor-binding site (Dey et al., 2009); unfortunately, while the CD4-induced antibodies do have broad reactivity with laboratory-adapted strains of HIV-1 that adopt an "open" conformation, they are generally unable to neutralize primary isolates (Labrijn et al., 2003). With MPER antibodies like 2F5, immunization with 2F5-epitope scaffolds can elicit antibodies capable of inducing the 2F5-bound conformation into peptides which otherwise prefer helical conformations (Ofek et al., 2010a); however, 2F5-epitope scaffold-elicited antibodies are unable to bind the 2F5 epitope in a membrane context and are unable to neutralize primary strains of HIV-1 (Ofek et al., 2010a). Similar results have been obtained with other MPER-epitope scaffolds (Correia et al., 2010) and constrained peptides (Bird et al., 2014), highlighting the need for co-recognition of membrane by MPER-directed antibodies (Irimia et al., 2016, 2017; Ofek et al., 2010b; Rujas et al., 2016). MPER-based design efforts are likely further hindered by tolerance mechanisms related to self-reactivity of some MPER directed antibodies, such as 2F5 and 4E10 (Haynes et al., 2005b; Verkoczy et al., 2017).

As the HIV-1 Env trimer is protected by multiple mechanisms of immune evasion, we and others have sought to test the concept of epitope-based vaccine design with other pathogens. Motavizumab (Synagis) is an antibody licensed for prevention of pneumonia caused by respiratory syncytial virus (RSV), the leading cause of hospitalization of infants. Motavizumab-epitope scaffolds elicited antibodies that bound the RSV F glycoprotein

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(McLellan et al., 2011a) or induced RSV-neutralizing antibodies (Correia et al., 2014), but neutralizing titers were generally low. Higher titers could be induced by immunizing with epitope scaffolds or stabilized subunit immunogens (Boyington et al., 2016; McLellan et al., 2013a), based on recognition by antibodies such as D25, AM14, and 5C4 specific for the prefusion conformation of RSV F with 10- to 100-fold higher neutralization potency than motavizumab (McLellan et al., 2013b). Such studies revealed the importance of choosing an appropriate target epitope for epitope-based vaccine design.

Epitope-Based Design for the CD4-Binding Site

Substantial efforts have been made toward the design of envelope immunogens based on the recognition of antibodies targeting the CD4-binding site of vulnerability, related to the functionally critical initial contact of HIV-1 with the CD4 receptor (Figure 4B; Zhou et al., 2007). Initial efforts involved the selection of immunogens that bound to broadly neutralizing antibodies such as antibody b12 (Burton et al., 1994), but not to antibodies like F105 (Posner et al., 1993), which were capable of neutralizing only laboratory-adapted isolates (Chen et al., 2009). These early efforts led to the engineering of outer domain-only immunogens (Joyce et al., 2013), conformationally stabilized core gp120s (Zhou et al., 2007), various envelope trimers (Farzan et al., 1998; Pancera et al., 2005; Yang et al., 2002), and glycan-masked immunogens (Ingale et al., 2014; Pantophlet et al., 2003). However, immunization with these proteins failed to elicit broadly neutralizing antibodies, likely because of conformational masking (Kwong et al., 2002), glycan shielding (Wei et al., 2003), and other mechanisms of immune evasion shielding this critical site (Chen et al., 2009).

The identification of multiple broadly neutralizing antibodies targeting the CD4-binding site has allowed for further refinement of immunogens. Moreover, the structure of a near-native closed trimer has revealed the degree that glycan and steric restrictions prevented antibody recognition of the CD4-binding site (Lyumkis et al., 2013; Stewart-Jones et al., 2016). These have inspired the creation of Env trimers with glycans pruned from the CD4-binding site, with improved recognition by broadly neutralizing antibodies, but not by non- or poorly neutralizing antibodies (Figure 4B); immunization with glycan-pruned Env immunogens elicits very high titers of antibodies against the CD4-binding site, though these are unable to neutralize wild-type viruses, and only glycan-pruned variants could be neutralized (Zhou et al., 2017). While additional immunization can elicit breadth in a subset of immunized animals, the titers of these have been less than optimal. Such difficulties in obtaining effective CD4-binding site antibodies parallels observations from HIV-1-infected donors, where CD4-binding site antibodies such as lineage CH235 take years to mature to effective neutralization, despite starting from a close to optimal angle of approach (Bonsignori et al., 2016).

Epitope-Based Design for the Glycan-V3 Site of Vulnerability

As noted above, analysis of sera from HIV-1-infected donors indicates the glycan-V3 category to be one of the most commonly targeted sites (Figure 4C; Georgiev et al., 2013; Landais et al., 2016; Pancera et al., 2014; Walker et al., 2010). Efforts to elicit antibodies that target this site range from glycan-rich yeast glycoproteins with 2G12 cross-reactivity (Agrawal-Gamse et al., 2011; Luallen et al., 2008; Zhang et al., 2015) to synthetic glycans or glycopeptides recognized by antibodies like PGT121 and PGT128 (Alam et al., 2017; Shivatare et al., 2018) to glycan-V3 epitope scaffolds (Zhou et al., 2014) or gp140 trimers (Saunders et al., 2017). Like other epitope efforts, the selection of immunogens has relied on recognition by broadly neutralizing antibodies and not by poor or non-neutralizing antibodies, such as those that target the protein component of the V3 loop.

While extensive immunizations have been attempted, to date none of these efforts have succeeded in reproducibly eliciting broadly reactive antibodies capable of neutralizing wild-type HIV-1. The yeast glycoprotein immunizations (Zhang et al., 2015) or gp140 immunizations (Saunders et al., 2017) have succeeded in eliciting antibodies capable of broad HIV-1 neutralization, though only to HIV-1 produced in the presence of the mannose-processing inhibitor kifunensine and restricted to have high mannose glycans. The substantial glycan component of the epitopes of antibodies that broadly neutralize at the glycan-V3 site is likely a primary reason that antibodies against this site have been recalcitrant to elicitation.

Epitope-Based Design for the Fusion Peptide Site of Vulnerability

Analysis of the epitopes of antibodies capable of neutralizing diverse primary isolates of HIV-1 indicates recognition of the prefusion-closed Env trimer to require recognition of glycan and to tolerate substantial sequence diversity (Chuang et al., 2018). Analysis of the Env segments that comprised each epitope reveals antibody VRC34.01 (Kong et al., 2016) to consist primarily of the exposed N-terminal eight residues (FP8) of the fusion peptide, a critical element of the HIV-1 entry machinery (Figure 4D). Xu et al. (2018) created a diverse panel of FP8-containing immunogens, ranging from FP8-epitope scaffolds to Env trimers and to FP8 chemically coupled to highly immunogenic carriers such as keyhole limpet hemocyanin (KLH). Assessment of these immunogens with broadly neutralizing antibodies VRC34.01 (Kong et al., 2016), PGT151 (Lee et al., 2016), and ACS202 (van Gils et al., 2016) indicated favorable antigenicity of both prefusion-stabilized Env trimers and FP8-KLH. Initial immunization with these immunogens elicited serum responses, capable of neutralizing only virus missing glycans such a N88 or N611, close to the fusion peptide. Iterative structure-based optimization, however, resulted in an immunization regimen—multiple FP8 and shorter peptide immunizations followed by Env trimer boosts—that elicits serum fusion peptide-directed neutralization in mice, guinea pigs, and rhesus macaques. Remarkably, fusion peptide-directed mAbs isolated from immunized mice display up to 31% breadth on a cross-clade panel of 208 HIV-1 strains (Xu et al., 2018). Both serum-neutralizing responses and mAbs induced by the FP8 immunization followed by Env trimer preferentially neutralize viral strains with the FP8 sequence used in immunization; as this sequence is relatively conserved, immunization with as few as three sequences may allow for neutralization breadths of up to 75%.

While breadth and titer of the immunogen-elicited fusion peptide-directed antibodies need to be improved, the elicited cross-clade breadth provides proof of principle for the generation of broadly neutralizing antibodies against HIV-1 by an

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epitope-based approach. It remains to be seen whether neutralization breadth of greater than 50% will require a combination of antibodies targeting different FP8 sequences or the appropriate maturation of broader lineages. Relevant to this, the complete functional mapping of the most effective vaccine-elicited murine antibodies (e.g., antibodies vFP16.02 and vFP20.01) indicates a focus on the N-terminal six amino acids of the fusion peptide (Dingens et al., 2018), with 10-fold increased concentration of vFP16.02 neutralizing 47% of a 208-strain cross-clade panel (Xu et al., 2018). We note that fusion peptide-directed antibodies are prevalent, but generally low titer in HIV-1-infected donors (Kong et al., 2016), and thus these fusion peptide-immunization results depend on the induction of much higher titers of antibodies against the fusion peptide than generally induced by HIV-1 infection.

Perspective: From Antibody to HIV-1 Vaccine

Over the last few years, an explosive growth in identifying broadly neutralizing antibodies against HIV-1 has yielded antibodies representative of hundreds of lineages. Characterization of the developmental pathways and structural modes of recognition of many of these antibodies has revealed diverse immunological mechanisms for achieving neutralization breadth along with atomic-level details from dozens of structurally characterized sites of vulnerability. Importantly, substantial strides have been made in the "antibody-to-vaccine" approach (Burton, 2002; Burton et al., 2004) to transform this wealth of immunological and structural information into effective vaccines.

With lineage-based vaccine design, antigens are designed to target specific ancestor B cells, with the goal of using sequential immunogens to induce appropriate affinity maturation of the antibody lineage and thus to generate cross-reactive neutralization (Figure 3). Antigen design can be informed by both the structural knowledge of an epitope (e.g., VRC01 class-immunogen design, Figure 3B) and by empirical testing of a library of antigens against UCA or early intermediate antibodies (e.g., PGT121 class, Figure 3C). The identification of reproducible or multidonor antibody classes, i.e., independent antibody lineages sharing genetic and structural characteristics, suggests that lineage-based design could be applied to target all of the template antibodies of a particular class (e.g., VRC01 class). Human gene knock-in mouse models have provided proof of principle that specific B cells can be targeted and expanded. However, there may be obstacles to successful lineage-based vaccine design and its translation into humans. These include the level of affinity maturation required for some antibody lineages to achieve effective neutralization (e.g., VRC01 class-antibody lineages), the insertions or deletions present in some broadly neutralizing antibody classes (e.g., PGT121 class), as well as the low inherent frequency of some B cell precursors, such as those encoding for an extended CDR H3 loop (e.g., PG9 class-antibody lineages). Thus, there is interest in identifying neutralizing antibodies with B cell ontogenies that may be less onerous to reproduce such as those for the CD4-binding site IOMA lineage (Chuang et al., 2018; Gristick et al., 2016) or for lineages with CDR H3 that are not too long with low to moderate SHM (MacLeod et al., 2016; Simonich et al., 2016). While transgenic mouse models can be useful in assessing and selecting vaccine antigens and immunization regimens, these models often overrepresent specific B cell populations and may therefore not accurately represent the human B cell repertoire. Mouse adoptive B cell transfer experiments are providing data on the roles of B cell precursor frequency as well as antigen affinity and avidity on B cell responses following immunization (Abbott et al., 2018), but critical information is needed from ongoing and future human phase I studies.

With epitope-based vaccine design (Figure 4), characteristics of appropriate target epitopes are now being delineated. Ideally, the target epitope immunogen would elicit a highly prevalent polyclonal (non-lineage-specific) response. Unfortunately, most epitopes on the HIV-1 Env trimer are less than ideal targets for such design, generally consisting of both protein and glycan (Gristick et al., 2016; Lee et al., 2016; Stewart-Jones et al., 2016) which are only semiconserved in amino acid sequence and in glycan location (e.g., N332 versus N334) (Chuang et al., 2018). In other cases, such as for V1V2 antibodies, B cell precursors may be rare (Briney et al., 2012a, 2012b; Doria-Rose et al., 2014; Walker et al., 2009). In yet other cases, precursor B cells or antibody responses may be limited by tolerance control mechanism (Doyle-Cooper et al., 2013; Haynes et al., 2005a; Verkoczy et al., 2017; Zhang et al., 2016). Thus, while numerous studies have tested Env subunit immunogens, and some elicit neutralizing antibodies to a limited number of HIV-1 strains, none have yet elicited cross-reactive serum neutralization to most primary strains of HIV-1 reviewed in (Burton and Mascola, 2015; Escolano et al., 2017). Initial success with the exposed N-terminal residues of the fusion peptide is, however, altering our notions of what can be achieved (Xu et al., 2018). This linear sequence of amino acids is relatively conserved and can be coupled to a carrier protein to increase its immunogenicity. When boosted by trimer immunization, cross-reactive fusion peptide-directed serum neutralization can be induced in some animals. Current immunization regimens are limited by both the consistency and potency of the elicited response—but the proof of principle for induction of broad serum neutralization responses is a foothold from which iterative structure-based optimization (Joyce et al., 2016b) will likely yield improvements.

While the progress of antibody-to-vaccine approaches is encouraging (Escolano et al., 2016; Xu et al., 2018), key next steps are to induce serum neutralization titers that can mediate protection in relevant challenge models. Here, passive antibody protection studies with SHIV challenge show a strong correlation between serum neutralization and protection (Pegu et al., 2017) and antibody correlates of protection are now being sought in humans (Gilbert et al., 2017). Will lineage-based vaccine design, based on a single class of template neutralizing antibody, succeed in inducing broad levels of protection? What levels of neutralization breadth can be achieved with epitope-based vaccine design? If only a few lineages are induced by epitope-based vaccine design, could lineage-based approaches assist by specifically priming and boosting vaccine-elicited lineages identified from epitope-based immunization? And to what level will other technologies such as nanoparticle presentation (Kanekiyo et al., 2013; Zhao et al., 2014) or gene-based immunization (Nabel, 2013) play a role? It seems likely that additional animal and phase 1 experimental medicine trials should be able to address most of these questions. For example, it will be critical to learn whether immunogens such at eOD-GT8 or 426c can induce VRC01 class antibody lineages in humans. Current

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animal and clinical trials testing the impact of passive antibody delivery are already revealing the power of mAbs—even showing full clearance, after virus has disseminated from the initial site of infection (Hessell et al., 2016; Liu et al., 2016).

Overall it appears that the antibody-to-vaccine approach is likely to be highly dependent on specificities of the antibody lineage or of the target epitope. It will be important to learn which broadly neutralizing antibody classes are the best templates for vaccines, and how this will differ for lineage-based and epitope-based approaches. The plethora of broad HIV-1-neutralizing antibodies identified as templates has clearly been critical to the success of antibody-to-vaccine approaches. It seems likely that similar approaches may work not only with HIV-1, but also for other pathogens, such as described above for epitope-based design with RSV. For protection against influenza, there is recent interest in inducing broadly reactive antibodies to the conserved stem region of hemagglutinin (HA)—similar to those defined by mAbs isolated from influenza-infected donors (Corti et al., 2011; Ekiert et al., 2009, 2011). This antibody-based vaccine approach takes advantage of immunization with diverse strains of HA, for which the head region is mismatched to prior infections but the stem region is conserved, thus focusing the immune response to the stem region of HA. This has resulted in reproducible (i.e., found in multiple donors) classes of anti-stem neutralizing antibodies that often derive from specific germline genes such as VH1-69, VH1-18, and VH6-1 (Joyce et al., 2016a; Kallewaard et al., 2016; Lingwood et al., 2012). These known antibody classes are now allowing specific lineage-based vaccine designs to induce anti-stem antibodies. With malaria, immunization with irradiated sporozoites (Seder et al., 2013) induces highly effective CSP-directed antibodies (Kisalu et al., 2018), some of which are similar to those found in vaccinees from malaria-endemic regions (Tan et al., 2018). Antibody-to-vaccine approaches reviewed here for HIV-1 may thus have efficacy with other pathogens for which standard approaches of vaccine development have failed.

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HHS-NIAID-20-0593-A-000159

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Wed, 16 May 2018 11:41:58 +0000
To: Stephanie Hayes
Cc: Conrad, Patricia (NIH/NIAID) [E];Billet, Courtney (NIH/NIAID) [E]
Subject: RE: Time-sensitive: Atlantic Magazine fact-checking questions

Stephanie:
Thanks for the note. I will respond to each of the questions in **red.**
Best regards,
Tony

From: Stephanie Hayes [mailto:shayes@theatlantic.com]
Sent: Monday, May 14, 2018 5:47 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: Time-sensitive: Atlantic Magazine fact-checking questions

Dr. Fauci,

I hope this email finds you well.

I'm a fact-checker at Atlantic Magazine in Washington, D.C, currently checking Ed Yong's article about pandemics for which you were interviewed a little while ago.

I'm writing in the hopes that you'd be willing to respond to a series of fact-checking questions—to confirm that the quotes and ideas we've attributed to you are correct, and that Ed's description of how a universal flu vaccine would be developed and would function is also accurate.

I've typed up a list of questions below—I'd be grateful if you could respond to these when you have a chance.

Please let me know if these questions require any clarification. And if you think it'd be easier to handle this over the phone, please just let me know when would suit you to speak.

Many thanks in advance,
Stephanie

1. Ed spells your name "Anthony Fauci" and identifies you as the director of the National Institute of Allergy and Infectious Diseases. Is that correct? **Correct**

2. Ed writes that preparing for a pandemic can seem like a nebulous task, but it ultimately boils down to real people and tangible things: A busy doctor raising an eyebrow when a patient presents with an unfamiliar fever. A nurse who takes a

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travel history. A hospital wing in which patients can be isolated. A warehouse where protective masks are stockpiled. A factory that churns out vaccines. A line on a budget. A vote in Congress.

3. Is that a fair claim (and not too reductive)? **It is a fair claim**

4. Following this, Ed writes that you told him: "It's like a chain—one weak link and the whole thing falls apart. You need no weak links." Is that correct? **Correct**

5. Speaking of how the US is preparing for a potential pandemic, Ed quotes you as saying: "We're chasing pre-pandemic. We have to do it," but the strategy remains wasteful and reactive. Is that correct? **It should read: "We occasionally have to chase pre-pandemics that threaten, but never materialize. We have to do it. But the strategy remains wasteful and reactive"**

6. Ed writes that you told him that what society really needs is a universal flu vaccine—one that protects against every variant of the virus, and provides long-term protection just as vaccines against measles and mumps do. Is that right? **Correct**

7. Ed writes that it's hard to overstate what a win a universal flu vaccine would be—no more worrying about strain mismatches or annual injections. Then, he quotes you as saying: "it would be the epitome of preparedness." Is that right? **Correct**

8. Ed explains that flu viruses are studded with a **protein** called haemagglutinin (the *H* in H1N1 and other such names), which, he writes, looks like a stumpy Pez dispenser. Is that an accurate description?

9. Ed writes that vaccines target the head of this molecule, but that's the part that varies the most between strains, and evolves most quickly. Is that correct? **I had to go to Google to find out what a Pez dispenser is. I did not use that metaphor. I felt that a more understandable metaphor would be a broccoli with the head and the stalk/stem or a mushroom with a cap and a stalk/stem.**

10. Ed writes that targeting the stem, which is more uniform and stable across strains, might yield better results. Is that right? **Correct**

11. Ed explains that the stem is, however, usually hidden from the immune system. Is that correct? **That is not entirely correct. It is more complicated than that. The head of the protein is "immunodominant" in that the immune system prefers to make a response to the head as opposed to the stem. One of the reasons is that the stem can be crowded out by the head; however, another reason is that the head just inherently induces a stronger immune response than does the stem**

12. Ed writes that to expose the stem, your team decapitate the molecule and stick the stem onto a nanoparticle. Is that right? **Correct**

AMERICAN OVERSIGHT

13.
14.
15. Ed writes that the result of this looks like a flu virus, but **since the immune system now only sees a stem (because the head is missing),** the immune system **makes a response against** the stable stem instead of the adaptable head. Is that an accurate description?

16. Ed explains that in a preliminary study, your team used this approach to build a vaccine using an **H1** virus, which then protected ferrets against a very different H5N1 strain. Is that right? **See minor correction**

17. Ed writes that this work is promising, but the flu is such an adaptable adversary that the quest for a universal vaccine might take years—or decades—to fulfill. Is that a fair claim? **Correct**

18. Ed writes that a universal-*ish* vaccine that, for example, protected against all H1N1 strains would have prevented the 2009 pandemic. Is that right? **Correct**

19. This seems fairly intuitive, but thought I'd run it past you anyway:
    Ed writes that taking even some variants of flu off the table would free up resources and intellectual capacity for dealing with other deadly diseases, for which no vaccines exist at all. Is that right? **Correct**

20. Ed writes that many of those deadly diseases for which no vaccines exist, strike poor countries first and are—for now, rare. Is that right? **Correct**

21. He adds that creating vaccines for them is painstaking and money-losing, so little gets done,. Is that a fair claim? **Not necessarily "Money-losing", but certainly not "profit-making", and so the government (NIH, BARDA) and non-profits often has to subsidize the development of such vaccines**

    Later on in the piece, Ed describes an exchange that took place in your office, so I wanted to run through the details he gives about that conversation.

    First off, he describes your office space:
22. Ed writes that your office walls are plastered with certificates, magazine articles, and other mementoes from your 34-year career as NIAID director—including photos of you with various presidents. Is that right? **Correct**

    b) to clarify, when people say the acronym for NIAID—do they pronounce it "ny-aid" or do they spell out each of the letters? **It goes both ways, but most people familiar with the Institute say Ny-ad**

23. Ed writes that in one picture on your wall, you stand in the Oval Office with Bill Clinton and Al Gore, pointing to a photo of HIV latching onto a white blood cell. Is that right? **Correct**

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24. He writes that in another photo, George W Bush fastens the Presidential Medal of Freedom around your neck. Is that correct? **Correct**

25. Ed writes that you have counseled every president since Ronald Reagan about the problem of epidemics, because every president has needed that counsel. He adds that you told him: "This transcends administrations." Is that right? **Correct**

26. Ed writes that you told him that the responses of presidents to epidemics varied. He writes that you told him that Clinton seemed indifferent, going on autopilot; the younger Bush made public health part of his legacy, funding an astonishingly successful anti-HIV program; Obama had the keenest intellectual interest in the subject. Is that right? **Correct with regard to Bush and Obama. Incorrect regarding Clinton. It was not that he was "indifferent". There really were not any major dramatic outbreaks during his Presidency such as Ebola, Zika, Anthrax, pandemic flu and so he did not get intimately involved with people like me in the context of infectious diseases. Besides, he had Donna Shalala as Secretary of HHS and she was very involved with us. And so, Ed must have gathered that I was inferring that Clinton was "indifferent". A better description, given the circumstances of little outbreak activity, I would say that he was less "on-hands' involved compared to Obama and Bush who had me to the Situation Room, Roosevelt Room, oval office multiple times.**

27. Ed writes that when he asked you about Trump you said: "I haven't had any interaction with him yet. But in fairness, there hasn't been a situation." Is that right? **Correct**

28. Ed writes that perhaps the two most important things a leader can personally provide in an epidemic are reliable information and a unifying spirit: In the absence of strong countermeasures, severe outbreaks tear communities apart, forcing people to fear their neighbors. Is that a fair claim, do you think? **Yes**

29. Ed writes that both catalyzing and organizing a federal response to an emergency pandemic is harder than one might think. He adds that the largely successful US response to Ebola in 2014 required the special appointment of an "Ebola czar" (Ron Klain) to array the disarray of overlapping agencies and unclear responsibility. Do you think that is a fair claim? **Ron Klain was great and helped coordinate responses of multiple agencies and made it easier for us to communicate with each other through him. I would not say at all that the agencies were in "disarray". That would be inaccurate. The coordination was much better with Ron in place, but we were not floundering.**

30. Finally, Ed writes that it is almost universally agreed that the best way of preventing pandemics is to contain outbreaks at their source, before they spread—the U.S. cannot possible consider itself protected if other nations are not. Is that a fair claim, do you think? **Correct.**

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31. Thanks, again.

--

Stephanie Hayes
Assistant Editor
The Atlantic

office: 202-266-7643
cell: (b)(6)

AMERICAN OVERSIGHT

From: Billet, Courtney (NIH/NIAID) [E]
Sent: Wed, 16 May 2018 10:58:51 +0000
To: Conrad, Patricia (NIH/NIAID) [E];Fauci, Anthony (NIH/NIAID) [E]
Subject: Re: ASM request for this mornings event

As an aside I don't know why she has the idea that you are giving a "talk" this morning. Just to reassure you that is NOT the expectation. You are going to be acknowledged by the Smithsonian and have the oppty to say a few words welcoming reporters. Per one pager i sent last week.

Sent from my iPhone

On May 16, 2018, at 6:05 AM, Conrad, Patricia (NIH/NIAID) [E] (b)(6) wrote:

FYI - pls see below. This is from the May 21 Stefano Bertuzzi event where you are showing slides. Event is at the newseum

Sent from my iPhone

Begin forwarded message:

From: "Khan, Aleea" (b)(6)
Date: May 15, 2018 at 4:27:45 PM EDT
To: "'Conrad, Patricia (NIH/NIAID) [E]'" (b)(6)
Cc: "Burmeister, Christina" (b)(6) "Vance, Meaghan (NIH/NIAID) [C]" (b)(6) "Eisinger, Robert (NIH/NIAID) [E]" (b)(6)
Subject: RE: Invitation to an event (May 21) in conjunction with the opening of the Outbreak exh

Dear Patty,

I know that Dr. Fauci will be speaking tomorrow at the Smithsonian's press event. I look forward to hearing his talk. Do you think he can mention that he will also be speaking on May 21 at ASM's event to celebrate the opening of "Outbreak," where new data will be released on the results of a national public opinion survey on vaccines and infectious disease outbreaks. I don't know if that fits in with what his focus is, but would love to promote to the audience that he is giving a talk on Monday as well.

Thank you,
Aleea

From: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Sent: Tuesday, May 15, 2018 2:03 PM
To: Khan, Aleea
Cc: Burmeister, Christina; Vance, Meaghan (NIH/NIAID) [C]; Eisinger, Robert (NIH/NIAID) [E]
Subject: RE: Invitation to an event (May 21) in conjunction with the opening of the Outbreak exh

Great – thank you all so much for making yet another change.

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This is now firm for 11:45 am tomorrow. Shall we use the same conference number?

Thanks again.

Patricia L. Conrad
Special Assistant to the Director

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From: Khan, Aleea (b)(6)
Sent: Tuesday, May 15, 2018 1:58 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Burmeister, Christina (b)(6)
Subject: RE: Invitation to an event (May 21) in conjunction with the opening of the Outbreak exh

Thanks for the heads-up. Stefano will be available to speak with Dr. Fauci at 11:45. I don't see it being longer than 10-15 minutes.

Best,
Aleea

From: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Sent: Tuesday, May 15, 2018 11:44 AM
To: Khan, Aleea
Subject: RE: Invitation to an event (May 21) in conjunction with the opening of the Outbreak exh

Hi Aleea

I am sorry but we may have to move the call. We may have an unforeseen change on Dr Fauci's calendar.

Who will be on the call for this? I am guessing it will not take more than about 15 minutes right? is it possible to do it at 11:45 am on May 16?

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If it is impossible to move – let me know. I am very sorry about this change but it's a bit out of our control.

Patricia L. Conrad
Special Assistant to the Director

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From: Khan, Aleea (b)(6)
Sent: Monday, May 14, 2018 2:49 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: Re: Invitation to an event (May 21) in conjunction with the opening of the Outbreak exh

Yes!

Aleea

On May 14, 2018, at 2:25 PM, Conrad, Patricia (NIH/NIAID) [E] (b)(6) wrote:

Will the May 16th date at 12:30 on ET work?

Patricia L. Conrad
Special Assistant to the Director

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Friday, May 11, 2018 1:34 PM
To: 'Khan, Aleea' (b)(6)
Cc: Burmeister, Christina (b)(6) Eisinger, Robert (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Subject: RE: Invitation to an event (May 21) in conjunction with the opening of the Outbreak exh

Can we do May 16th at 12:30 pm ET?

Patricia L. Conrad

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Special Assistant to the Director

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From: Khan, Aleea (b)(6)
Sent: Friday, May 11, 2018 12:54 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Burmeister, Christina (b)(6)
Subject: RE: Invitation to an event (May 21) in conjunction with the opening of the Outbreak exh

Please let me know what time and date works for him and we can schedule a call with him.

Thank you,
Aleea

From: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Sent: Friday, May 11, 2018 12:53 PM
To: Khan, Aleea
Subject: RE: Invitation to an event (May 21) in conjunction with the opening of the Outbreak exh

Dr Fauci is not able to join the call. He would prefer a call you all at another date and time – he understands if the other participants are not on the call. Is that possible to schedule?

Patricia L. Conrad
Special Assistant to the Director

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From: Khan, Aleea (b)(6)
Sent: Thursday, May 10, 2018 2:44 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: RE: Invitation to an event (May 21) in conjunction with the opening of the Outbreak exh

Dear Patty,

AMERICAN OVERSIGHT

We are aiming for a **1pm call on May 15th** with all the speakers at the event to go over the talks of the day. This shouldn't be a long call, just a quick overview. Will Dr. Fauci be available at that time? If not, can someone else listen in on his behalf?

Thank you!
Aleea

From: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Sent: Tuesday, May 01, 2018 4:15 PM
To: Khan, Aleea
Subject: RE: Invitation to an event (May 21) in conjunction with the opening of the Outbreak exh

Do you know who the CDC speaker is yet?

Patricia L. Conrad
Special Assistant to the Director

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From: Khan, Aleea (b)(6)
Sent: Tuesday, May 01, 2018 3:36 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: RE: Invitation to an event (May 21) in conjunction with the opening of the Outbreak exh

Dear Patty,

I had a meeting with Stefano who wants Dr. Fauci's focus to be a bit broader. It would be great if he could tell the story of where we were in terms of preparedness during the 1918 pandemic, then discuss the 2009 H1N1 outbreak and compare that to today. After that, he can talk more specifically about the strategic plan for the universal flu vaccine.

A lot of that is what is discussed here can be reiterated: https://www.nih.gov/news-events/news-releases/dynasty-influenza-virus-1918-today.

He wants Dr. Fauci to have a bigger role, in speaking about preparedness/surveillance and paint the overall picture of how far we have come and what the next steps are in containing outbreaks.

Thank you!
Aleea

From: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, April 25, 2018 12:05 PM

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To: Khan, Aleea
Subject: RE: Invitation to an event (May 21) in conjunction with the opening of the Outbreak exh

For Dr. Fauci – do you mean he should only cover universal flu or do you want a broader talk on vaccines and then narrow down to universal flu?

Patricia L. Conrad
Special Assistant to the Director

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From: Khan, Aleea (b)(6)
Sent: Wednesday, April 25, 2018 11:42 AM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6) Bertuzzi, Stefano (b)(6)
Cc: Vance, Meaghan (NIH/NIAID) [C] (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6) Ort-Mabry, Catherine (b)(6) Lontok, Katherine (b)(6) Eisinger, Robert (NIH/NIAID) [E] (b)(6)
Subject: RE: Invitation to an event (May 21) in conjunction with the opening of the Outbreak exh

Dear Patty,

Please see a draft agenda for the event below. We are still working on the details, but I hope you find this helpful as Dr. Fauci prepares his remarks. We are still waiting on confirmation on a CDC speaker.

What: The American Society for Microbiology (ASM) will host a briefing with Research!America in collaboration with the American Society for Virology (ASV) that will:

- Celebrate the opening of "Outbreak: Epidemics in a Connected World." This Smithsonian National Museum of Natural History exhibit marks the anniversary of the Great Influenza pandemic and will open on May 18. ASM is proud to be teaming up with The Smithsonian in this effort.

- Release the results of a national public opinion survey on vaccines and infectious disease outbreaks. Survey findings will generate new insights and highlight trends on public perception of vaccines and outbreaks.

When: May 21 at 10 a.m. -11:30 a.m. ET

Where: Newseum
555 Pennsylvania Ave NW,

AMERICAN OVERSIGHT

Washington, DC 20001

Who: Speakers and agenda of the event:

- **Opening remarks and moderator (5 minutes)**
  Stefano Bertuzzi, CEO, ASM
- **Brief remarks (5 minutes)**
  Stacey Schultz-Cherry, President, ASV
- **Survey results and highlights (15 minutes)**
  Mary Woolley, President, Research!America
- **Status of vaccine research and a strategic plan for developing a universal influenza vaccine (20 minutes)**
  Anthony Fauci, Director, National Institute of Allergy and Infectious Diseases, NIH
- **Public health priorities regarding infectious disease preparedness/response (20 minutes)**
  CDC spokesperson
- **Q&A (10-15 minutes)**

Please let me know if you have an additional questions.

Best,
Aleea Khan
Senior Manager, Science Communications
ASM

From: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, April 25, 2018 11:07 AM
To: Bertuzzi, Stefano
Cc: Vance, Meaghan (NIH/NIAID) [C]; Folkers, Greg (NIH/NIAID) [E]; Khan, Aleea; Ort-Mabry, Catherine; Lontok, Katherine; Eisinger, Robert (NIH/NIAID) [E]
Subject: RE: Invitation to an event (May 21) in conjunction with the opening of the Outbreak exh

Good morning:

Can you please send us any details as to Dr. Fauci's role at the event….will this be a lecture or panel? We just need to know as Dr. Fauci would like to prepare his remarks/slides or whatever is required. Any info and details would be appreciated.

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Many thanks,

-patty

Patricia L. Conrad
Special Assistant to the Director

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From: Bertuzzi, Stefano (b)(6)
Sent: Tuesday, April 24, 2018 7:53 AM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Vance, Meaghan (NIH/NIAID) [C] (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6) Khan, Aleea (b)(6) Ort-Mabry, Catherine (b)(6) (b)(6) Lontok, Katherine (b)(6)
Subject: Re: Invitation to an event (May 21) in conjunction with the opening of the Outbreak exh

Excellent. This is confirmed/

Best
S

Stefano Bertuzzi, Ph.D., M.P.H.
Chief Executive Officer
American Society for Microbiology (ASM)
1752 N St., NW
Washington, DC 20036-2904
Phone (b)(6)

From: "Conrad, Patricia (NIH/NIAID) [E]" (b)(6)
Date: Tuesday, April 24, 2018 at 7:44 AM
To: Stefano Bertuzzi (b)(6)
Cc: "Vance, Meaghan (NIH/NIAID) [C]" (b)(6) "Folkers, Greg (NIH/NIAID) [E]" (b)(6) Aleea Khan (b)(6) Catherine Ort-Mabry (b)(6) (b)(6) Katherine Lontok (b)(6)
Subject: RE: Invitation to an event (May 21) in conjunction with the opening of the Outbreak exh

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Dr Fauci is still available on May 21st from 10 am – 11:30 am.

Thank you.

Patricia L. Conrad
Special Assistant to the Director

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From: Bertuzzi, Stefano (b)(6)
Sent: Tuesday, April 24, 2018 2:37 AM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Vance, Meaghan (NIH/NIAID) [C] (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6) Khan, Aleea (b)(6) Ort-Mabry, Catherine (b)(6) (b)(6) Lontok, Katherine (b)(6)
Subject: Re: Invitation to an event (May 21) in conjunction with the opening of the Outbreak exh

Dear Pat—

After a few back and forth trying to accommodate several things, the best possible day/time for the ASM event with Researarch!America on the opening of the Outbreak exhibit is **May 21st from 10:00 to 11:30** at the Newseum. We are still waiting for final confirmation from Anne Schuchat from CDC, but we expect a positive answer, based on our conversations.

I hope Dr. Fauci is still available in this window of time. Thank you for your patience with scheduling.

Best regards,
S

Sent from the iPhone of
Stefano Bertuzzi, Ph.D., M.P.H.
Chief Executive Officer
American Society for Microbiology (ASM)
1752 N St., NW
Washington, DC 20036-2904
Phone (b)(6)
Twitter @sutefune

On Apr 17, 2018, at 10:36 PM, Conrad, Patricia (NIH/NIAID) [E] (b)(6) wrote:

AMERICAN OVERSIGHT

Sorry for the delay in getting back to you. Dr. Fauci has a rather long meeting that day and will be unavailable from about 11 am to 3 pm. In addition, the timing of this meeting could change - - meaning it might change on either end. We are pretty much holding the day for this meeting since it is such a big chunk of time.

If there is another day other than May 15th – it might be better to look at that if possible.

-patty

Patricia L. Conrad
Special Assistant to the Director

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From: Bertuzzi, Stefano (b)(6)
Sent: Monday, April 16, 2018 6:38 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Subject: Re: Invitation to an event (May 21) in conjunction with the opening of the Outbreak exh

Dear Pat —

We had to move the event to May 15, as I mentioned the 21st was a place holder. I still do not have a location and a time, but it will likely be at the National Press Club and around breakfast time. Given that this is the day before the opening of the exhibit, we think we will have more coverage, unfortunately, we will not have the private tour of the exhibit.

I should be able to confirm more details tomorrow, but in the meantime, it would be great to know if Dr. Fauci would be available on the 15th.

Thank you,
S

Stefano Bertuzzi, Ph.D., M.P.H.
Chief Executive Officer
American Society for Microbiology (ASM)

AMERICAN OVERSIGHT

1752 N St., NW
Washington, DC 20036-2904
Phone (b)(6)

From: "Conrad, Patricia (NIH/NIAID) [E]" (b)(6)
Date: Thursday, April 12, 2018 at 3:53 PM
To: Stefano Bertuzzi (b)(6)
Cc: "Vance, Meaghan (NIH/NIAID) [C]" (b)(6)
Subject: FW: Invitation to an event (May 21) in conjunction with the opening of the Outbreak exhibit

Dr. Fauci is happy to do this – we are holding May 21 at 10 am.

-patty

Patricia L. Conrad
Special Assistant to the Director

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From: Bertuzzi, Stefano (b)(6)
Sent: Wednesday, April 11, 2018 5:58 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Schwetz, Tara (NIH/NIAID) [E] (b)(6)
Subject: Invitation to an event (May 21) in conjunction with the opening of the Outbreak exhibit

Dear Tony —

I hope all is well. I am reaching out regarding an event that ASM is planning with Research!America in conjunction with the opening of the *Outbreak* exhibit at the Museum of Natural History. As you may know, ASM partnered with the Smithsonian on the exhibit and we are committed to several outreach events.

The first one may be on May 21st at 10:00 AM at the Newseum (still to be confirmed). The idea is to have you and the new CDC Director as featured speakers. We will also release a survey that R!A and us will carry out on vaccines. The target audience of the event is Congress, the Washington crowd of policy folks, and the press.

The event is still to be finalized, but I wanted to check in with you now to see if you have interest in participating, and if you happen to be available on May 21st (we are waiting to hear also from Dr. Redfield).

Thank you for considering, I hope you can make it.

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Best wishes,
Stefano

Stefano Bertuzzi, Ph.D., M.P.H.
Chief Executive Officer
American Society for Microbiology (ASM)
1752 N St., NW
Washington, DC 20036-2904
Phone (b)(6)

AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Tue, 15 May 2018 16:27:53 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: 2:15 pm - Call with Devex Michael Igoe re 15th Anniv of PEPFAR
Attachments: RE: PEPFAR 15th Anniversary

AMERICAN OVERSIGHT

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Tue, 15 May 2018 16:19:26 +0000
To: Michael Igoe
Cc: Vance, Meaghan (NIH/NIAID) [C]
Subject: RE: PEPFAR 15th Anniversary

Actually – can we do 2:15 pm ET? That will give him some time to get back to the office from another meeting. Thanks.

Patricia L. Conrad
Special Assistant to the Director

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From: Michael Igoe <michael.igoe@devex.com>
Sent: Tuesday, May 15, 2018 12:14 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: Re: PEPFAR 15th Anniversary

Hi Patty,

That works. My number is (b)(6) I'll call at 2pm.

Much appreciated, Michael

Sent from my iPhone

On May 15, 2018, at 11:56 AM, Conrad, Patricia (NIH/NIAID) [E] (b)(6) wrote:

Hi Michael:
Dr. Fauci can be available at 2 pm ET today. IF that works – please call (b)(6)

Please send us your cell as back up as well.

Many thanks,
-patty

AMERICAN OVERSIGHT

Patricia L. Conrad
Special Assistant to the Director

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Monday, May 14, 2018 7:35 PM
To: Michael Igoe <michael.igoe@devex.com>
Cc: Conrad, Patricia (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Subject: Re: PEPFAR 15th Anniversary

Michael:
Thank you for your note. I would be delighted to chat with you about the 15th anniversary of PEPFAR. I am getting ready to get on an (b)(6) We can chat this evening (b)(6) (b)(6) Just let me know. If that does not work for you, please give my Special Assistant, Patty Conrad, a call tomorrow at (b)(6) or (b)(6) to set a mutually convenient time for us to chat.
Best regards,
Tony

Sent from my iPad

On May 14, 2018, at 6:14 PM, Michael Igoe <michael.igoe@devex.com> wrote:

Dear Dr. Fauci,

I hope this finds you well. My name is Michael Igoe. I'm a reporter with Devex. I interviewed you last year about pandemic security for this story: https://www.devex.com/news/q-a-with-tony-fauci-on-the-state-of-pandemic-security-89799

I'm reaching out now because we're putting together some coverage for the 15th anniversary of PEPFAR. The first article is asking how PEPFAR changed global health (beyond just the injection of money), and we're anchoring the story with several key interviews (Mark Dybul, Ambassador Birx, etc).

I was wondering if there was any chance you'd be available in the next week or so to speak with me about this topic. I'm happy to share more details and answer any questions if this sounds possible.

Many thanks,

Michael

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--

Michael Igoe
Senior Reporter

Devex – Do Good. Do It Well.™

(b)(6) (m)

@AlterIgoe

Devex is the media platform for the global development community.

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From: Stephanie Hayes
Sent: Mon, 14 May 2018 17:47:05 -0400
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Time-sensitive: Atlantic Magazine fact-checking questions

Dr. Fauci,

I hope this email finds you well.

I'm a fact-checker at Atlantic Magazine in Washington, D.C, currently checking Ed Yong's article about pandemics for which you were interviewed a little while ago.

I'm writing in the hopes that you'd be willing to respond to a series of fact-checking questions—to confirm that the quotes and ideas we've attributed to you are correct, and that Ed's description of how a universal flu vaccine would be developed and would function is also accurate.

I've typed up a list of questions below—I'd be grateful if you could respond to these when you have a chance.

Please let me know if these questions require any clarification. And if you think it'd be easier to handle this over the phone, please just let me know when would suit you to speak.

Many thanks in advance,
Stephanie

1. Ed spells your name "Anthony Fauci" and identifies you as the director of the National Institute of Allergy and Infectious Diseases. Is that correct?

2. Ed writes that preparing for a pandemic can seem like a nebulous task, but it ultimately boils down to real people and tangible things: A busy doctor raising an eyebrow when a patient presents with an unfamiliar fever. A nurse who takes a travel history. A hospital wing in which patients can be isolated. A warehouse where protective masks are stockpiled. A factory that churns out vaccines. A line on a budget. A vote in Congress. Is that a fair claim (and not too reductive)?

3. Following this, Ed writes that you told him: "It's like a chain—one weak link and the whole thing falls apart. You need no weak links." Is that correct?

4. Speaking of how the US is preparing for a potential pandemic, Ed quotes you as saying: "We're chasing a pre-pandemic. We have to do it," but the strategy remains wasteful and

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reactive. Is that correct?

5. Ed writes that you told him that what society really needs is a universal flu vaccine—one that protects against every variant of the virus, and provides long-term protection just as vaccines against measles and mumps do. Is that right?

6. Ed writes that it's hard to overstate what a win a universal flu vaccine would be—no more worrying about strain mismatches or annual injections. Then, he quotes you as saying: "it would be the epitome of preparedness." Is that right?

7. Ed explains that flu viruses are studded with a molecule called haemagglutinin (the *H* in H1N1 and other such names), which, he writes, looks like a stumpy Pez dispenser. Is that an accurate description?

8. Ed writes that vaccines target the head of this molecule, but that's the part that varies the most between strains, and evolves most quickly. Is that correct?

9. Ed writes that targeting the stem, which is more uniform and stable across strains, might yield better results. Is that right?

10. Ed explains that the stem is, however, usually hidden from the immune system. Is that correct?

11. Ed writes that to expose the stem, your team decapitate the molecule and stick the stem onto a nanoparticle. Is that right?

12. Ed writes that the result of this looks like a flu virus, but trains the immune system to go after the stable stem instead of the adaptable head. Is that an accurate description?

13. Ed explains that in a preliminary study, your team used this approach to build a vaccine using an H1N1 virus, which then protected ferrets against a very different H5N1 strain. Is that right?

14. Ed writes that this work is promising, but the flu is such an adaptable adversary that the quest for a universal vaccine might take years—or decades—to fulfill. Is that a fair claim?

15. Ed writes that a universal-*ish* vaccine that, for example, protected against all H1N1 strains would have prevented the 2009 pandemic. Is that right?

16. This seems fairly intuitive, but thought I'd run it past you anyway:
    Ed writes that taking even some variants of flu off the table would free up resources and

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intellectual capacity for dealing with other deadly diseases, for which no vaccines exist at all. Is that right?

17. Ed writes that many of those deadly diseases for which no vaccines exist, strike poor countries first and are—for now, rare. Is that right?

18. He adds that creating vaccines for them is painstaking and money-losing, so little gets done,. Is that a fair claim?

Later on in the piece, Ed describes an exchange that took place in your office, so I wanted to run through the details he gives about that conversation.

First off, he describes your office space:

19. Ed writes that your office walls are plastered with certificates, magazine articles, and other mementoes from your 34-year career as NIAID director—including photos of you with various presidents. Is that right?

b) to clarify, when people say the acronym for NIAID—do they pronounce it "ny-aid" or do they spell out each of the letters?

20. Ed writes that in one picture on your wall, you stand in the Oval Office with Bill Clinton and Al Gore, pointing to a photo of HIV latching onto a white blood cell. Is that right?

21. He writes that in another photo, George W Bush fastens the Presidential Medal of Freedom around your neck. Is that correct?

22. Ed writes that you have counseled every president since Ronald Reagan about the problem of epidemics, because every president has needed that counsel. He adds that you told him: "This transcends administrations." Is that right?

23. Ed writes that you told him that the responses of presidents to epidemics varied. He writes that you told him that Clinton seemed indifferent, going on autopilot; the younger Bush made public health part of his legacy, funding an astonishingly successful anti-HIV program; Obama had the keenest intellectual interest in the subject. Is that right?

24. Ed writes that when he asked you about Trump you said: "I haven't had any interaction with him yet. But in fairness, there hasn't been a situation." Is that right?

25. Ed writes that perhaps the two most important things a leader can personally provide in an epidemic are reliable information and a unifying spirit: In the absence of strong countermeasures, severe outbreaks tear communities apart, forcing people to fear their

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neighbors. Is that a fair claim, do you think?

26. Ed writes that both catalyzing and organizing a federal response to an emergency pandemic is harder than one might think. He adds that the largely successful US response to Ebola in 2014 required the special appointment of an "Ebola czar" (Ron Klain) to array the disarray of overlapping agencies and unclear responsibility. Do you think that is a fair claim?

27. Finally, Ed writes that it is almost universally agreed that the best way of preventing pandemics is to contain outbreaks at their source, before they spread—the U.S. cannot possible consider itself protected if other nations are not. Is that a fair claim, do you think?

Thanks, again.

--

Stephanie Hayes
Assistant Editor
The Atlantic

office: 202-266-7643
cell: (b)(6)

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From: Billet, Courtney (NIH/NIAID) [E]
Sent: Mon, 14 May 2018 20:31:13 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: RE: universal flu vaccine

ok

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Monday, May 14, 2018 4:28 PM
To: Billet, Courtney (NIH/NIAID) [E] (b)(6)
Subject: Fwd: universal flu vaccine

NIAID inquiries, please

Begin forwarded message:

From: Maria Ines Azambuja (b)(6)
Date: May 14, 2018 at 3:51:13 PM EDT
To: (b)(6)
Subject: universal flu vaccine

Dear Dr. Fauci

I was just reading about the Universal Flu vaccine idea. You say: "It's a scientific challenge because there are still so many things we don't know," says Anthony Fauci, director of the NIAID."
https://www.scientificamerican.com/article/a-new-push-for-a-universal-flu-vaccine/?utm_source=newsletter&utm_medium=email&utm_campaign=daily-digest&utm_content=link&utm_term=2018-05-14_featured-this-week&spMailingID=56613585&spUserID=MjQwNTIwNTI4NTYS1&spJobID=1402109815&spReportId=MTQwMjEwOTgxNQS2

I believe that this is very true.
We don´t know enough so maybe we should not attempt to do this.

I believe that we have co-evolved with influenza viruses and that as they change, the human population changes, what would explain the secular trends in several causes of mortality and morbidity...

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Influenza may be a good thing. It may have contributed to differentiated us according to birth cohorts, what would prevent us to die all at the same time due to generalized vulnerability to some external agent.

Imagine an epidemic in a chicken farm, all chicken made equal.
And now think in our worst pandemic - the black death? 25-30% mortality?

Nature is wise and I would not undue what I don´t understand...

Best regards

Maria Inês Azambuja, MD

Azambuja MI 2009. Influenza recycling and secular trends in mortality and natality.
https://www.researchgate.net/publication/222093732_Influenza_Recycling_and_Secular_Trends_in_Mortality_and_Natality

Prof. Adjunta
Departamento de Medicina Social
Faculdade de Medicina da
Universidade Federal do Rio Grande do Sul

Rua Ramiro Barcelos, 2600, 4/420
90035-003, Porto Alegre, RS
(b)(6)

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Mon, 14 May 2018 20:11:50 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Bullets to look at for your train ride home
Attachments: Press Preview for Outbreak.docx

FYI – you are doing 2 events for the Smithsonian Outbreak Exhibit on May 16. The morning one will have you give 4-5 minutes of remarks and then meet with press that are there to view the exhibit. Courtney prepared the attached bullets. Please let me know if these are ok. thought you could look at these on your way home on the train

thx

Patricia L. Conrad
Special Assistant to the Director

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Friday, May 11, 2018 2:16 PM
To: Vance, Meaghan (NIH/NIAID) [C] (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6)
Subject: FW: From NIAID/NIH: Press and Social Media Collaborations for "Outbreak: Epidemics in a Connected World"

Fyi – 4-5 mins of remarks.

Patricia L. Conrad
Special Assistant to the Director

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From: Kremer, Randall (b)(6)
Sent: Friday, May 11, 2018 2:15 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Billet, Courtney (NIH/NIAID) [E] (b)(6) Torres, Anna K. (b)(6)
Subject: RE: From NIAID/NIH: Press and Social Media Collaborations for "Outbreak: Epidemics in a Connected World"

Correct. 4-5 minutes will be fine. Thanks!

From: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Sent: Friday, May 11, 2018 2:14 PM
To: Kremer, Randall (b)(6)
Cc: Billet, Courtney (NIH/NIAID) [E] (b)(6) Torres, Anna K. (b)(6)
Subject: RE: From NIAID/NIH: Press and Social Media Collaborations for "Outbreak: Epidemics in a Connected World"

Thank you so much. We will aim to have Dr. Fauci arrive at 9 am and depart at approximately 10 am. Just to clarify – we should prepare for his remarks to be no more than 5 minutes.

Thank you and best regards,
-patty

Patricia L. Conrad
Special Assistant to the Director

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From: Kremer, Randall (b)(6)
Sent: Friday, May 11, 2018 2:06 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Billet, Courtney (NIH/NIAID) [E] (b)(6) Torres, Anna K. (b)(6)
Subject: RE: From NIAID/NIH: Press and Social Media Collaborations for "Outbreak: Epidemics in a Connected World"

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Hello Patty - We are delighted that Dr. Fauci can join us for the press preview for Outbreak, next Wednesday, May 16 (advisory attached). The preview is from 9:30am-12:30pm, with brief remarks from Kirk Johnson, Sabrina Sholts and Dr. Fauci between 9:45 and 10am. Dr. Fauci is welcome to join us earlier if his schedule allows. Some of the collaborators will be arriving between 9:00 - 9:15am to view the exhibition. Mr. Fauci should arrive at the (b)(6) (b)(6) There will staff there to escort him up to the exhibition.

Please let me know if that covers everything. We are hoping that you and Courtney can join us for the preview.

Best,

Randall Kremer

Randall Kremer
Director of Public Affairs
National Museum of Natural History
Smithsonian Institution, MRC-135
10th St. & Constitution Ave., NW
Washington, D.C. 20560-0135
Tel: (b)(6) Cell: (b)(6)

From: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Sent: Friday, May 11, 2018 1:52 PM
To: Kremer, Randall (b)(6)
Cc: Billet, Courtney (NIH/NIAID) [E] (b)(6)
Subject: RE: From NIAID/NIH: Press and Social Media Collaborations for "Outbreak: Epidemics in a Connected World"

Good afternoon –

Am just touching base to see if you had any details to pass along.

Many thanks,
-patty

Patricia L. Conrad
Special Assistant to the Director

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Tuesday, May 08, 2018 11:34 AM
To: (b)(6) (b)(6)
Subject: FW: From NIAID/NIH: Press and Social Media Collaborations for "Outbreak: Epidemics in a Connected World"

Hi Randall:

I am the POC for Dr. Fauci only – please send us the details for the event at your earliest convenience. Please include arrival time, location, onsite contact name and numbers and any other details regarding Dr. Fauci's role at your event.

Thanks and I look forward to working with you on this.

Best,
-patty

Patricia L. Conrad
Special Assistant to the Director

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From: Billet, Courtney (NIH/NIAID) [E]
Sent: Tuesday, May 08, 2018 11:30 AM
To: Kremer, Randall (b)(6)
Cc: Tennessen, Tina (b)(6) Torres, Anna K. (b)(6) Fabry, Cynthia (NIH/NIAID) [E] (b)(6) Marquardt, Julie (NIH/NIAID) [E] (b)(6) Stover, Kathy (NIH/NIAID) [E] (b)(6) Morens, David (NIH/NIAID) [E] (b)(6) Taubenberger, Jeffery (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Subject: RE: From NIAID/NIH: Press and Social Media Collaborations for "Outbreak: Epidemics in a Connected World"

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I'm so sorry for doing this piecemeal, but with Dr. Fauci now also a "yes" for the morning press preview, I wanted to add his assistants Patty Conrad and Meaghan Vance to this chain, as points of contact for logistical arrangements.

Thanks Randall!

From: Billet, Courtney (NIH/NIAID) [E]
Sent: Monday, May 07, 2018 12:19 PM
To: 'Kremer, Randall' (b)(6)
Cc: 'Tennessen, Tina' (b)(6) 'Torres, Anna K.' (b)(6) Fabry, Cynthia (NIH/NIAID) [E] (b)(6) Marquardt, Julie (NIH/NIAID) [E] (b)(6) Stover, Kathy (NIH/NIAID) [E] (b)(6) Morens, David (NIH/NIAID) [E] (b)(6) Taubenberger, Jeffery (NIH/NIAID) [E] (b)(6)
Subject: RE: From NIAID/NIH: Press and Social Media Collaborations for "Outbreak: Epidemics in a Connected World"

We have a yes from Jeff Taubenberger as well! Jeff and David are an amazing duo. I've added Jeff to this chain so the right folks on your end can be in contact with him.

From: Billet, Courtney (NIH/NIAID) [E]
Sent: Friday, May 04, 2018 5:27 PM
To: 'Kremer, Randall' (b)(6)
Cc: 'Tennessen, Tina' (b)(6) 'Torres, Anna K.' (b)(6) Fabry, Cynthia (NIH/NIAID) [E] (b)(6) Marquardt, Julie (NIH/NIAID) [E] (b)(6) Stover, Kathy (NIH/NIAID) [E] (b)(6) Morens, David (NIH/NIAID) [E] (b)(6)
Subject: RE: From NIAID/NIH: Press and Social Media Collaborations for "Outbreak: Epidemics in a Connected World"

Me again. I just went ahead and checked in with Drs. Morens, Taubenberger, and Mascola about their availability. I have already heard back from Dr. Morens - and I am so happy that he is able to participate. He is both incredibly knowledgeable and totally engaging – a perfect match for the task at hand!

I have copied Dr. Morens so that the appropriate people on your end can be in touch with him directly about particulars.

From: Billet, Courtney (NIH/NIAID) [E]
Sent: Friday, May 04, 2018 5:04 PM
To: 'Kremer, Randall' (b)(6)
Cc: Tennessen, Tina (b)(6) Torres, Anna K. (b)(6) Fabry, Cynthia (NIH/NIAID) [E] (b)(6) Marquardt, Julie (NIH/NIAID) [E] (b)(6) Stover, Kathy (NIH/NIAID) [E] (b)(6)

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Subject: RE: From NIAID/NIH: Press and Social Media Collaborations for "Outbreak: Epidemics in a Connected World"

Hi Randall—sorry we've missed each other!

Is the plan for the press preview still as we discussed a few weeks ago? Essentially, a very brief welcome from the Smithsonian, and then the reporters are free to walk through the exhibit and interact with SME's who are essentially serving as docents? That sounds like an opportunity well-suited to Drs. Taubenberger, Morens, and possibly Mascola (his schedule is especially challenging), all of whom are I think well-known to the exhibit organizers. To clarify: have the organizers already invited them directly?

As far as Dr. Fauci goes, he is definitely attending the reception in the evening, but our understanding was that the morning event would be rather informal and better suited for the SME's. If something has changed and you're now planning on a more formal program in the morning, I could see what is possible, though it could be challenging at this point.

In any event, we are all very much looking forward to the exhibit!

From: Kremer, Randall (b)(6)
Sent: Thursday, May 03, 2018 1:20 PM
To: Billet, Courtney (NIH/NIAID) [E] (b)(6)
Cc: Tennessen, Tina (b)(6) Torres, Anna K. (b)(6)
Subject: RE: From NIAID/NIH: Press and Social Media Collaborations for "Outbreak: Epidemics in a Connected World"

Hi Courtney – We would be thrilled to have Dr. Fauci at the press preview, as well as Dr. Taubenberger, Dr. Mascola and Dr. Morens. I am available for a follow up chat at your convenience.

Best,

Randall Kremer

Randall Kremer
Director of Public Affairs
National Museum of Natural History
Smithsonian Institution, MRC-135
10th St. & Constitution Ave., NW
Washington, D.C. 20560-0135
Tel: (b)(6) Cell: (b)(6)

AMERICAN OVERSIGHT

Press Preview for *Outbreak: Epidemics in a Connected World*
May 16, 2018

Background:
The "Outbreak" exhibit is opening at the Smithsonian's National Museum of Natural History and will run for 3 years, receiving up to 10 million visitors.

You have been invited to the press preview. You will arrive a bit early to have the chance to walk through the exhibit yourself, ahead of reporters. Once reporters arrive, there will be a brief welcome from Smithsonian leadership and they will recognize you to say a few welcoming/laudatory words, very informally (4-5 minutes), which could include:

- Reiterating the exhibit's objectives (see below)
- Noting "the perpetual challenge" of emerging/ re-emerging infectious diseases
- Mentioning any aspects of the exhibit that particularly struck you
- Commending the Smithsonian and their collaborators
- Acknowledging the role of the media in educating the public on science and health

Following the welcoming remarks, reporters will be free to walk through the exhibit. There will be subject matter experts scattered throughout, to answer questions. David Morens and Jeff Taubenberger are attending in this capacity.

Exhibit Objectives:
The Exhibit's objectives are for audiences to understand that:

- Human health, animal health, and environmental health are intimately connected;
- Effective outbreak responses are often multidisciplinary and international;
- Outbreaks have broad, and sometimes unexpected, societal consequences; and
- There are specific actions and a range of careers needed to prevent and control infectious disease outbreaks.

Four viruses receive special focus: influenza, HIV/AIDS, Zika, Ebola

NMNH will offer a second, "pop-up" version of the exhibit for communities to print and display in venues around the world, thus extending its impact.

NIAID Contributions:

- $25K
- Personal perspectives, subject matter expertise (Fauci, Morens, Taubenberger, Mascola)
- Numerous images and 3-D models

Other contributors include Rockefeller Foundation, Lyda Hill Foundation, Johnson & Johnson Innovation, Open Philanthropy Project, Skoll Global Threats Fund, Page Family Foundation Donor Advised Fund, Seqirus, Anders Foundation, National Foundation for Infectious Diseases, Centers for Disease Control and Prevention, Infectious Diseases Society of America, RTI International, EcoHealth Alliance, ProMED/International Society for Infectious Diseases, the USAID Emerging Pandemic Threats Division and the American Society for Microbiology.

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Mon, 14 May 2018 14:27:26 +0000
To: Fauci, Anthony (NIH/NIAID) [E];McDermott, Adrian (NIH/VRC) [E];Paules, Catharine (NIH/NIAID) [E]
Subject: Discuss commentary —"Immunity to Influenza: A Moving Target" for The Journal of Immunology
Attachments: RE: Commentary for the Journal of Immunology

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From: Thompson, Danielle (NIH/NIAID) [V]
Sent: Mon, 14 May 2018 14:19:55 +0000
To: Vance, Meaghan (NIH/NIAID) [C]
Cc: McDermott, Adrian (NIH/VRC) [E]
Subject: RE: Commentary for the Journal of Immunology

Good Morning Meaghan,

Adrian's availability is noted below.

Please let us know your preference.

Thanks,
Danielle

From: Vance, Meaghan (NIH/NIAID) [C]
Sent: Friday, May 11, 2018 9:19 AM
To: Thompson, Danielle (NIH/NIAID) [V] (b)(6)
Cc: McDermott, Adrian (NIH/VRC) [E] (b)(6)
Subject: RE: Commentary for the Journal of Immunology

Will any of these options work?

May 23rd 4pm – **available**
May 24th 11-1:30pm – **not available**
May 25th 10-4pm – **available 11:30-4pm**

From: Thompson, Danielle (NIH/NIAID) [V]
Sent: Thursday, May 10, 2018 1:15 PM
To: Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Cc: McDermott, Adrian (NIH/VRC) [E] (b)(6)
Subject: Commentary for the Journal of Immunology

Dear Meaghan,

Please provide available options for the week of May 21st when you have a chance.

Thanks,

Danielle Thompson
Assistant to:
Dr. Richard A. Koup, Deputy Director, Immunology Lab
Dr. Adrian McDermott, Chief, Vaccine Immunology Program
Dr. Costas Petrovas, Head, Tissue Analysis Core
Vaccine Research Center

AMERICAN OVERSIGHT

40 Convent Drive, MSC 3022
Bldg. 40 Room 3504
Bethesda, MD 20892
Tel. (b)(6)
Fax 301-480-2779

From: Paules, Catharine (NIH/NIAID) [E]
Sent: Thursday, May 10, 2018 12:32 PM
To: McDermott, Adrian (NIH/VRC) [E] (b)(6)
Cc: Thompson, Danielle (NIH/NIAID) [V] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: RE: Commentary for the Journal of Immunology

Thanks very much Adrian! Looking forward to working with you. Meaghan Vance will be in touch to coordinate with Dr. Fauci's calendar. Safe travels, Cat

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From: McDermott, Adrian (NIH/VRC) [E]
Sent: Thursday, May 10, 2018 12:24 PM
To: Paules, Catharine (NIH/NIAID) [E] (b)(6)
Cc: Thompson, Danielle (NIH/NIAID) [V] (b)(6)
Subject: Re: Commentary for the Journal of Immunology

Dear Cat,

Of course I can assist with this. Pleasure to work with you on it.

Let me know a good time we can chat. I am out of the country next week but available after that.

Thanks

Adrian

Sent from my iPhone

On May 10, 2018, at 12:12 PM, Paules, Catharine (NIH/NIAID) [E] (b)(6) wrote:

Dear Adrian,
Dr. Fauci asked me to reach out to you to see if you might be interested in working with us, as a co-author, on a commentary for a topical review issue—"Immunity to Influenza: A Moving Target" for *The Journal of Immunology*. The commentary would be approximately 2000 words and would need to be submitted by September 1, 2018. I would do the bulk of the drafting but would much appreciate your expertise on framing the manuscript and editing. If you are interested Dr. Fauci would like to set up a brief meeting to discuss in the next week or two. Thanks very much for considering, Cat

AMERICAN OVERSIGHT

From: Paules, Catharine (NIH/NIAID) [E]
Sent: Mon, 14 May 2018 14:32:43 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Accepted: Discuss commentary —"Immunity to Influenza: A Moving Target" for The Journal of Immunology

AMERICAN OVERSIGHT

From: STEWART SIMONSON
Sent: Mon, 14 May 2018 14:05:53 +0200
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Lane, Cliff (NIH/NIAID) [E]
Subject: Re: PRIVATE—Ebola Care and Treatment

Ok. Thanks for this, Tony. I really appreciate it.

Sent from my iPhone

On 14 May 2018, at 2:03 PM, Fauci, Anthony (NIH/NIAID) [E] (b)(6) wrote:

Stewart:
Absolutely yes! We would be happy to serve as a receiving hospital for exposed/infected WHO staff.
Cliff:
We should also check with Lu Borio at NSC to make sure DHS or some other agency does not block this.
Best,
Tony

On May 14, 2018, at 7:47 AM, STEWART SIMONSON (b)(6) wrote:

Thanks a million, Tony. One related item: if—God forbid—one of our [WHO] folks is exposed in this outbreak and needs to be hospitalized, do you think the NIH would want to be considered as a possible receiving hospital? I am putting our plans together since we are deploying quite a few people to DRC.

Sent from my iPhone

On 14 May 2018, at 11:59 AM, Fauci, Anthony (NIH/NIAID) [E] (b)(6) wrote:

Stewart:
No problem at all. We would be happy to do this. Rick Davey can take the lead. I will discuss with Cliff. Look forward to seeing you in (b)(6)
Thanks,
Tony

From: STEWART SIMONSON (b)(6)
Sent: Monday, May 14, 2018 5:00 AM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: Ebola Care and Treatment

AMERICAN OVERSIGHT

Dear Tony:

Hope all is well. I am flailing around getting ready for the World Health Assembly, which should be something…. Cliff was here last week and I think he had a pretty good experience with the first meeting of new board of scientific counselors to the WHO emergencies program. So that is a step in the right direction.

One of my responsibilities here is the medical program for staff. So we have physicians and nurses, etc. in Geneva and around the world. Since you treated ebola patients at (b)(6) and know the disease. I was wondering if you, Cliff and some of the folks from the Special Clinical Studies Unit would consider participating in a video conference with our staff here—probably about 50 or 60 folks maybe more The objective is to make sure everyone here who may be deployed is up-to-date on how to treat ebola, etc. Soumya and I would likely host it on our end. This is something the staff want so I would like to help make it happen if possible. Probably no more than an hour or so.

I will be back in June for a few weeks, I hope. And will look forward to seeing Christine and you then.

Stewart

STEWART SIMONSON
Washington, DC/Geneva, Switzerland
(b)(6) **or**
(b)(6)
(b)(6) **(US)**
(b)(6) **(Switzerland)**

AMERICAN OVERSIGHT

From: Marston, Hilary (NIH/NIAID) [E]
Sent: Sun, 13 May 2018 23:36:08 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Lane, Cliff (NIH/NIAID) [E];Handley, Gray (NIH/NIAID) [E]
Subject: Re: inquiry from INRB

Great – thank you.

From: Anthony Fauci (b)(6)
Date: Sunday, May 13, 2018 at 7:24 PM
To: Hilary Marston (b)(6)
Cc: Henry Lane (b)(6) Gray Handley (b)(6)
Subject: RE: inquiry from INRB

(b)(5)

Thanks,
Tony

From: Marston, Hilary (NIH/NIAID) [E]
Sent: Sunday, May 13, 2018 4:21 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: Fwd: inquiry from INRB

Do you have a moment to discuss?
(b)(6)

Begin forwarded message:

From: PREZIOSI, Marie-pierre (b)(6)
Date: May 13, 2018 at 5:11:21 AM EDT
To: Marston, Hilary (NIH/NIAID) [E] (b)(6) HENAO RESTREPO, Ana Maria (b)(6)
Cc: FORMENTY, Pierre B.h. (b)(6) Fauci, Anthony (NIH/NIAID) [E] (b)(6) Lane, Cliff (NIH/NIAID) [E] (b)(6) Higgs, Elizabeth (NIH/NIAID) [E] (b)(6) Handley, Gray (NIH/NIAID) [E] (b)(6) Mascola, John (NIH/VRC) [E] (b)(6) Graham, Barney (NIH/VRC) [E] (b)(6) SATHIYAMOORTHY, Vaseeharan (b)(6) DIAZ, Janet Victoria (b)(6)
Subject: RE: inquiry from INRB

Dear Hilary,

AMERICAN OVERSIGHT

Thank you very much for sharing the protocol and the two publications.

(b)(5)

Kind regards,

Marie-Pierre

From: Marston, Hilary (NIH/NIAID) [E] (b)(6)
Sent: 13 May 2018 01:12
To: HENAO RESTREPO, Ana Maria
Cc: FORMENTY, Pierre B.h.; Fauci, Anthony (NIH/NIAID) [E]; Lane, Cliff (NIH/NIAID) [E]; Higgs, Elizabeth (NIH/NIAID) [E]; Handley, Gray (NIH/NIAID) [E]; Mascola, John (NIH/VRC) [E]; Graham, Barney (NIH/VRC) [E]; SATHIYAMOORTHY, Vaseeharan; PREZIOSI, Marie-pierre
Subject: RE: inquiry from INRB

Dear Ana Maria,

(b)(5)

Warm regards,

Hilary

AMERICAN OVERSIGHT

From: HENAO RESTREPO, Ana Maria (b)(6)
Sent: Saturday, May 12, 2018 1:40 AM
To: Marston, Hilary (NIH/NIAID) [E] (b)(6)
Cc: Pierre Formenty (b)(6) Fauci, Anthony (NIH/NIAID) [E] (b)(6) Lane, Cliff (NIH/NIAID) [E] (b)(6) Higgs, Elizabeth (NIH/NIAID) [E] (b)(6) Handley, Gray (NIH/NIAID) [E] (b)(6) Mascola, John (NIH/VRC) [E] (b)(6) Graham, Barney (NIH/VRC) [E] (b)(6) SATHIYAMOORTHY, Vaseeharan (b)(6) PREZIOSI, Marie-pierre (b)(6)
Subject: Re: inquiry from INRB

Dear Hillary,

Many thanks for your kind message.
Indeed we are trying to encourage partners to collaborate and also gather the expert advice from all.

This information is very useful and many thanks for taking this action.

Colleagues are preparing an " overall plan" regarding the potential use of various experimental therapeutics.

In this regard, if you could please share with us any detailed information regarding mAb114 and any protocol available for -expanded access and for a trial-will be an excellent first steps.

A series of teleconferences are planned this week and we trust you and the other NIAID experts will join them. Details will be send on Monday.

Kind regards

Ana María

Sent from my iPhone

On 12 May 2018, at 00:09, Marston, Hilary (NIH/NIAID) [E] (b)(6) wrote:

Dear Ana Maria –

(b)(5)

Warm regards and please feel free to contact me with questions,

AMERICAN OVERSIGHT

Hilary

Hilary Marston, MD, MPH
Medical Officer and Policy Advisor for Global Health
NIAID Office of the Director
(b)(6)
(b)(6) (cell)

AMERICAN OVERSIGHT

From: Mascola, John (NIH/VRC) [E]
Sent: Sun, 13 May 2018 18:46:38 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Lane, Cliff (NIH/NIAID) [E];Graham, Barney (NIH/VRC) [E];Marston, Hilary (NIH/NIAID) [E];Ledgerwood, Julie (NIH/NIAID) [E]
Subject: FW: follow up from our call
Attachments: Corti et al mAb114.pdf, Misasi et al mAb114.pdf, (b)(5)
(b)(5)

Forwarding FYI. Email follow up from Julie after phone call discussion yesterday with Muyembe and colleagues at INRB.

From: Ledgerwood, Julie (NIH/NIAID) [E]
Sent: Saturday, May 12, 2018 5:01 PM
To: Mulangu Sabue (b)(6) Jean Jacques Muyembe-Tamfum (b)(6) Olivier TSHIANI (b)(6)
Cc: Graham, Barney (NIH/VRC) [E] (b)(6) Mascola, John (NIH/VRC) [E] (b)(6) Sullivan, Nancy (NIH/VRC) [E] (b)(6)
Subject: follow up from our call

Dear Professor, Sabue and Olivier

We appreciated the call and chance to talk with you this morning. We know how very busy you are at this time.

From our conversation this morning we understood that the DRC MoH will likely send a formal request for the Ebola mAb114 product which was co-discovered by your group at the INRB in Kinshasa from a Congolese Ebola survivor.

We also appreciated hearing that the WHO DRC representatives are supportive of your plans and request. Our understanding is that in addition to keeping WHO informed, you will also let us know about any regulatory process needed regarding import permits.

As you know, this product has not been administered to any humans as yet, but is scheduled to be administered to the first Phase 1 healthy subject in the US study within the next week, and could possibly be provided now for compassionate use if requested. Using the product for treatment as early as possible after infection is supported by the preclinical studies in which macaques survived if given a single dose of mAb114 up to 5-6 days after infection, even after early symptomatic disease is evident.

As we discussed, I have included the Phase 1 protocol for the US trial in healthy subjects as well as the Investigator's Brochure and a summary of preparation information that could be informative. I have also attached the 2 Science publications from 2016 that describe the biology and preclinical characteristics of the antibody.

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Please let us know if you have any questions for us.

Best regards,
Julie

Julie Ledgerwood, D.O.
Chief
Clinical Trials Program
Vaccine Research Center
NIAID, NIH
(b)(6)
(b)(6)

AMERICAN OVERSIGHT

EBOLA VIRUS

Protective monotherapy against lethal Ebola virus infection by a potently neutralizing antibody

Davide Corti,[1,2]* John Misasi,[3]* Sabue Mulangu,[3] Daphne A. Stanley,[3] Masaru Kanekiyo,[3] Suzanne Wollen,[4] Aurélie Ploquin,[3] Nicole A. Doria-Rose,[3] Ryan P. Staupe,[3] Michael Bailey,[3] Wei Shi,[3] Misook Choe,[3] Hadar Marcus,[3] Emily A. Thompson,[3] Alberto Cagigi,[3] Chiara Silacci,[1] Blanca Fernandez-Rodriguez,[1] Laurent Perez,[1] Federica Sallusto,[1] Fabrizia Vanzetta,[2] Gloria Agatic,[2] Elisabetta Cameroni,[2] Neville Kisalu,[5]† Ingelise Gordon,[3] Julie E. Ledgerwood,[3] John R. Mascola,[3] Barney S. Graham,[3] Jean-Jacques Muyembe-Tamfun,[5] John C. Trefry,[4]‡ Antonio Lanzavecchia,[1,6]‡ Nancy J. Sullivan[3]‡§

Ebola virus disease in humans is highly lethal, with case fatality rates ranging from 25 to 90%. There is no licensed treatment or vaccine against the virus, underscoring the need for efficacious countermeasures. We ascertained that a human survivor of the 1995 Kikwit Ebola virus disease outbreak maintained circulating antibodies against the Ebola virus surface glycoprotein for more than a decade after infection. From this survivor we isolated monoclonal antibodies (mAbs) that neutralize recent and previous outbreak variants of Ebola virus and mediate antibody-dependent cell-mediated cytotoxicity in vitro. Strikingly, monotherapy with mAb114 protected macaques when given as late as 5 days after challenge. Treatment with a single human mAb suggests that a simplified therapeutic strategy for human Ebola infection may be possible.

Ebola virus disease (EVD) causes severe illness characterized by rapid onset of fever, vomiting, diarrhea, and bleeding diathesis (*1*, *2*). The challenges of a large outbreak and the failure of traditional quarantine and contact-tracing measures (*3*, *4*) to control the 2014 West Africa outbreak highlight the urgent need for therapies. The success in nonhuman primates (NHP) of ZMapp—a cocktail of three chimeric monoclonal antibodies (mAbs) derived from immunized mice (*5*–*7*)—illustrated the potential of mAb therapies against EVD, and ZMapp is currently being evaluated in human trials. To date, efforts in NHP to simplify the ZMapp regimen to contain fewer mAbs have not been successful (*7*). We sought to isolate mAbs from human EVD survivors, with the goal of identifying antibodies that confer clinical protection either as single or dual-combination agents.

We obtained blood from two survivors of the 1995 EVD outbreak in Kikwit, Democratic Republic of the Congo (*8*), 11 years after infection. To determine whether the subjects retained circulating antibodies against Ebola virus (EBOV) glycoprotein (GP), we assessed GP-specific antibodies by enzyme-linked immunosorbent assay (ELISA) (Fig. 1A) (*9*). The reciprocal 10% maximal binding EC_{90} titer (the reciprocal dilution at which there is a 90% decrease in antigen binding) for subject 1 (S1) was 2326, higher than control sera by more than a factor of 10. Moreover, serum from the more severely ill subject, S1, displayed potent virus-neutralizing activity (Fig. 1B); this finding indicates that S1 maintained serologic memory against EBOV GP more than a decade after infection and suggested the potential for cloning immunoglobulins with potent neutralizing activity from S1's memory B cells.

Therefore, we sorted memory B cells from S1's peripheral blood mononuclear cells and immortalized individual clones with Epstein-Barr virus (*10*). Forty clone supernatants displayed a range of GP binding (Fig. 1C); two of them, 100 and 114,

[1]Institute for Research in Biomedicine, Università della Svizzera Italiana, CH-6500 Bellinzona, Switzerland. [2]Humabs BioMed SA, 6500 Bellinzona, Switzerland. [3]Vaccine Research Center, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892, USA. [4]U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD 21702, USA. [5]National Institute for Biomedical Research, National Laboratory of Public Health, Kinshasa B.P. 1197, Democratic Republic of the Congo. [6]Institute of Microbiology, ETH Zürich, CH-8093 Zürich, Switzerland.
*These authors contributed equally to this work. †Present address: Vaccine Research Center, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892, USA. ‡These authors contributed equally to this work. **§Corresponding author. E-mail: njsull@mail.nih.gov**

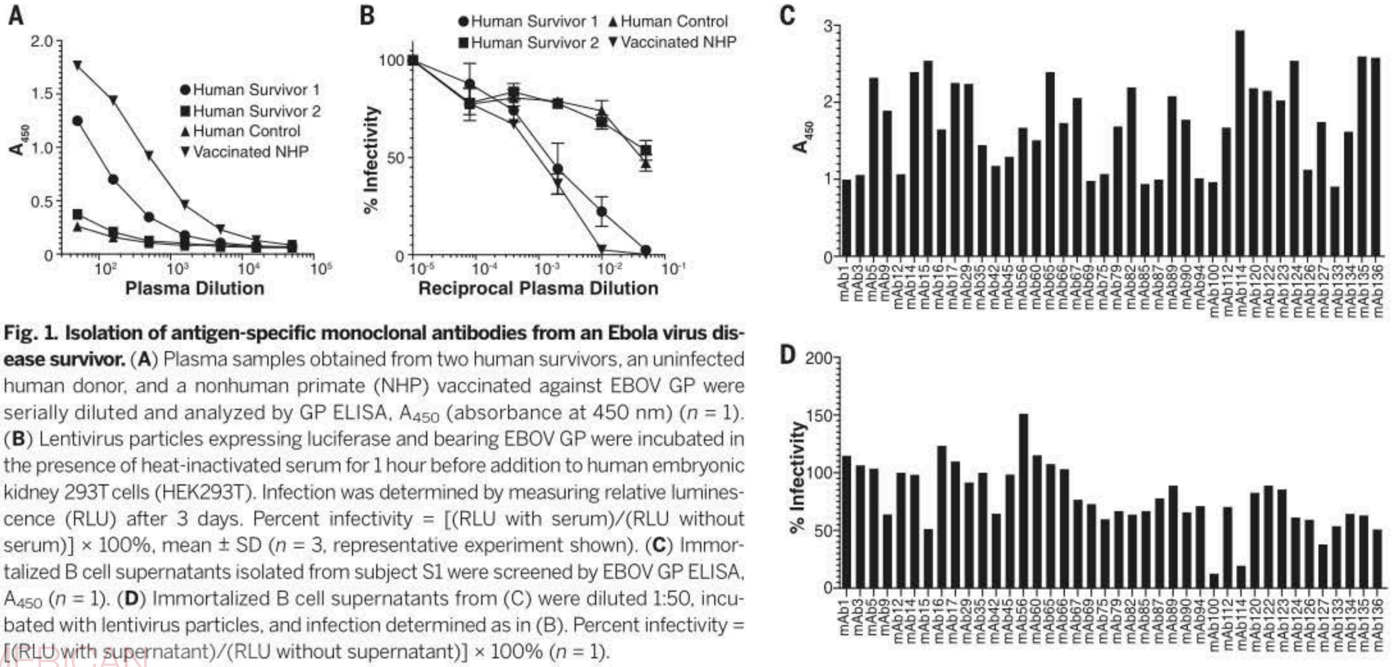

Fig. 1. Isolation of antigen-specific monoclonal antibodies from an Ebola virus disease survivor. (**A**) Plasma samples obtained from two human survivors, an uninfected human donor, and a nonhuman primate (NHP) vaccinated against EBOV GP were serially diluted and analyzed by GP ELISA, A_{450} (absorbance at 450 nm) (n = 1). (**B**) Lentivirus particles expressing luciferase and bearing EBOV GP were incubated in the presence of heat-inactivated serum for 1 hour before addition to human embryonic kidney 293T cells (HEK293T). Infection was determined by measuring relative luminescence (RLU) after 3 days. Percent infectivity = [(RLU with serum)/(RLU without serum)] × 100%, mean ± SD (n = 3, representative experiment shown). (**C**) Immortalized B cell supernatants isolated from subject S1 were screened by EBOV GP ELISA, A_{450} (n = 1). (**D**) Immortalized B cell supernatants from (C) were diluted 1:50, incubated with lentivirus particles, and infection determined as in (B). Percent infectivity = [(RLU with supernatant)/(RLU without supernatant)] × 100% (n = 1).

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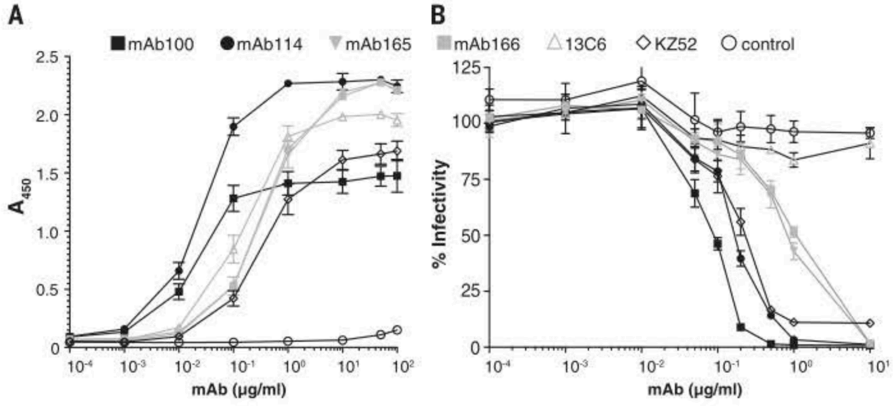
expressed antibodies with markedly higher neutralizing activity than all others (Fig. 1D). A second immortalization yielded 21 clones, from which the GP-specific clones 165 and 166 were rescued (fig. S1).

mAb100, mAb114, mAb165, and mAb166 sequences were amplified by polymerase chain reaction (PCR) and antibodies produced by transient transfection. We assessed ELISA binding to EBOV GP and observed that mAb114, mAb165, and mAb166 displayed maximal binding nearly 50% higher than that of KZ52, a prototypic EBOV GP–specific human mAb (*11*), and 25% higher than that of 13C6, a component of the ZMapp cocktail (*6*, *7*) (Fig. 2A). The binding curve of mAb100 had a plateau similar to that of KZ52 (Fig. 2A). mAb100 and mAb114 achieved half-maximal binding (EC_{50}) at a concentration of 0.02 μg/ml, which is lower than the other mAbs by a factor of 7 to 19. The binding profiles of mAb165 and mAb166 were similar to each other, with EC_{50} values of 0.38 μg/ml and 0.35 μg/ml, respectively; EBOV control mAbs KZ52 and 13C6 had EC_{50} values of 0.33 μg/ml and 0.14 μg/ml, respectively (Fig. 2A).

To evaluate the four S1 mAbs' capacity for neutralization, we used lentiviral particles pseudotyped with EBOV GP, a method that has been demonstrated to recapitulate wild-type EBOV results without the need for high-level biocontainment procedures (*12*) (Fig. 2B and fig. S2A). mAb165 and mAb166 exhibited similar half-maximal inhibition (IC_{50}) concentrations of 1.77 and 0.86 μg/ml, respectively. mAb100 and mAb114 were the most potent, with IC_{50} values about one log unit lower (0.06 and 0.09 μg/ml, respectively) than those of mAb165 and mAb166. Notably, all four of the isolated mAbs inhibited 100% of the virus, unlike KZ52, which consistently displayed only 80 to 90% maximum inhibition, and 13C6, which neutralized <20% at 10 μg/ml. The S1 mAbs also potently neutralized the 2014 West African Makona variant (fig. S2B). Neutralization of wild-type EBOV particles by each of the isolated antibodies was confirmed by plaque reduction assay (fig. S3).

Sequence analysis revealed that the S1 mAbs displayed 85 to 95% germline identity for heavy chains and 89 to 97% germline identity for light chains (Fig. 2C). Analyses of germline gene usage and V(D)J recombination indicate that they originated from different B cell lineages. We analyzed the role of somatic hypermutations for the two most potent antibodies, mAb100 and mAb114, with the use of variants that were partially or completely reverted to the unmutated common ancestors (UCAs) (Fig. 2D and fig. S4, A and B). The fully reverted mAb100 (UCA/UCA), as well as a variant with a single change from the UCA VL (UCA/gL, $Ala^{89} \rightarrow Thr$), recognized cells expressing GP, with binding weaker than that of the fully matured antibody by a factor of 2 to 4 (Fig. 2E and fig. S4, A and B). When three HCDR3 (heavy chain complementarity-determining region 3) mutations—$Ala^{96} \rightarrow Val$, $Val^{103} \rightarrow Tyr$, and $Tyr^{114} \rightarrow Ser$—were introduced in the reverted germline antibody (gH/UCA), binding was comparable to the fully matured mAb100 (sH/sL); this result suggests that three mutations are sufficient to confer the binding observed for the fully matured mAb100.

In the case of mAb114, the fully reverted version (UCA/UCA) demonstrated negligible binding



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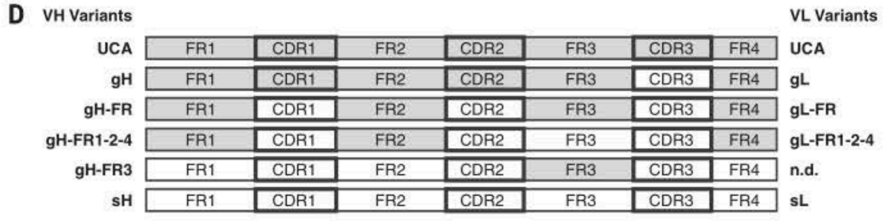
	VH	Identity %	D	JH	VL	Identity %	JL	HCDR3 length	IgG subclass
mAb100	V4-59*01	94.74%	D3-16*02	J4*02	VL3-1*01	92.11%	J2*01	19	IgG3
mAb114	V3-13*01	87.37%	D6-19*01	J4*02	VK1-27*01	92.83%	J4*01	13	IgG1
mAb165	V3-7*01	89.93%	D2-2*02	J4*02	VK1-39*01	89.96%	J1*01	21	IgG1
mAb166	V1-69*04	92.01%	D3-22*01	J6*03	VK3-20*01	97.16%	J3*01	16	IgG3



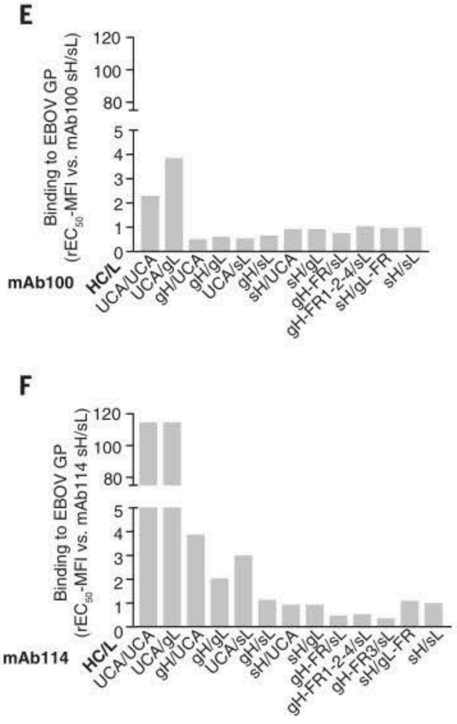


Fig. 2. Characterization of purified EBOV GP monoclonal antibodies. (**A**) EBOV GP ELISA in the presence of purified mAbs as indicated: A_{450}, mean ± SD (n = 3, representative experiment shown). (**B**) Pseudotyped EBOV GP lentivirus particles were incubated with increasing amounts of purified mAbs and infection of HEK293T cells determined as in Fig. 1B. Percent infectivity = [(RLU with antibody)/(RLU without antibody)] × 100%, mean ± SD (n = 3, representative experiment shown). (**C**) V-gene usage, sequence analysis, and immunoglobulin G (IgG) subclass of antibodies from S1. (**D**) Schematic of mAb100 and mAb114 unmutated common ancestor (UCA) and variants created for investigation of the binding requirements of these mAbs. Shaded areas represent sequence from UCA; light regions are from the somatic, mature antibody. Wild-type, somatically mutated heavy (sH), or somatically mutated light (sL) chains; gH or gL, germline V-gene revertants of sH or sL in which the HCDR or LCDR3 (light chain complementarity-determining region 3) are mature; gH-FR or gL-FR, germline V-gene revertants of sH or sL in which the HCDRs or LCDRs are mature; gH-FR1-2-4, germline V-gene revertants of sH in which the HCDRs and HFR3 (framework region 3 of heavy chain) are mature; gH-FR3, germline V-gene revertants of sH in which the HCDRs and HFR1, HFR2, and HFR4 are mature. (**E** and **F**) Binding to EBOV GP expressed on the surface of Madin-Darby canine kidney-2,6-sialtransferase (MDCK-SIAT) cells by versions of mAb100 (E) and mAb114 (F) in which all or subsets of somatic mutations were reverted to the germline sequence. Shown is the ratio between the EC_{50} values of the variants and the wild type (sH/sL). Ratio values above 100 indicate a lack of detectable binding (n = 1).

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to GP (Fig. 2F and fig. S4, C and D). Introduction of two mutations (Ala^{96} → Val and Tyr^{108} → Ser) in the HCDR3 of the mAb114 germline (gH/UCA) was sufficient to confer an increase in binding, although still not to the degree seen with the fully matured mAb. Because these mutations are located at the base of the HCDR3 loop, they likely do not make direct contact with GP and thus may have a stabilizing effect on the whole HCDR3. The fully matured light chain and the two HCDR3 mutations (gH/sL) were sufficient to confer binding equivalent to the fully matured mAb (sH/sL). The fully mutated light chain gene (UCA/sL) could partially compensate for a lack of somatic mutation in the heavy chain (Fig. 2F and fig. S4, C and D). The presence of additional mutations on either VH or VL is required to achieve the level of the fully matured mAb114 binding. These results suggest a rapid pathway of mAb114 affinity maturation through one or two somatic mutations, which became redundant as further mutations accumulated—a finding that is reminiscent of what was recently observed for the generation of broadly neutralizing influenza antibodies (*13*).

Because mAb100 and mAb114 were the most potently neutralizing antibodies, they were considered optimal candidates for further evaluation in NHP. To assess the potential for combination therapy, we first sought to rule out antagonistic binding to GP. We found that each antibody bound to GP in the presence of the other, which suggests that they recognize distinct regions on GP (Fig. 3A) and therefore could be used as combination immunotherapy to maximize efficacy (*14*). To define the regions targeted by mAb100 and mAb114, we used biolayer interferometry to assess GP binding in competition with mAbs KZ52 and 13C6, which have epitopes in the GP base and glycan cap, respectively (*15*, *16*). We found that mAb100 competes with KZ52 for binding at the base of GP, whereas mAb114 recognizes (at least in part) the glycan cap region, as demonstrated by competition with 13C6 (Fig. 3, B and C).

Because some EBOV GP antibodies have been suggested to mediate antibody-dependent cell-mediated cytotoxicity (ADCC) (*17*), we determined the ADCC activity of mAb100 and mAb114 in a flow cytometric assay (Fig. 3D). We found that mAb100 and mAb114 mediated ADCC, with maximal activity observed at a mAb concentration of 0.03 μg/ml. Target cell killing was mediated through Fc receptors, because mAbs containing Fc LALA mutations [Leu^{234} → Ala, Leu^{235} → Ala (*18*)] abrogated ADCC activity. Therefore, these mAbs have the potential to induce direct killing of infected cells in vivo, a key viral clearance mechanism.

The presence of potent neutralizing and ADCC activity and the absence of cross-competition supported testing whether mAb100 and mAb114 offered in vivo protective efficacy. We challenged four rhesus macaques with a lethal dose of EBOV. One day after challenge, the treatment group (n = 3) received an intravenous injection with a mixture of mAb100 and mAb114 at a combined dose of 50 mg/kg, and the treatment was repeated twice at 24-hour intervals. Circulating GP-specific antibody titers in mAb recipients peaked after the second injection, and reciprocal ELISA titers remained above 10^5 throughout the study, suggesting minimal clearance of the mAbs (Fig. 4A). The untreated macaque succumbed to EVD on day 10 with a circulating viral load exceeding 10^8 genome equivalents per milliliter (ge/ml) (Fig. 4, B and C). In contrast, all three mAb-treated macaques survived challenge without detectable viremia. Consistent with historical controls, the untreated animal displayed hallmark indicators of EVD including thrombocytopenia and elevations in alanine transaminase and creatinine from day 6 through the time of death (Fig. 4D and figs. S5 to S8). In contrast, the treatment group remained within normal ranges for these parameters and remained free of all EVD symptoms.

We next asked whether monotherapy is sufficient for protection, focusing on mAb114 because it showed higher maximal binding than mAb100. We exposed four macaques to a lethal dose of EBOV and administered mAb114 (50 mg/kg; n = 3) to the treatment group after a 1-day delay, followed by two doses at 24-hour intervals. All treated macaques survived, whereas the control animal succumbed to EVD on day 6 with a peak viral load of 10^{10} ge/ml (Fig. 4, E to G). In contrast to the previous experiment, transient viremia was observed in the treated animals (Fig. 4F) but

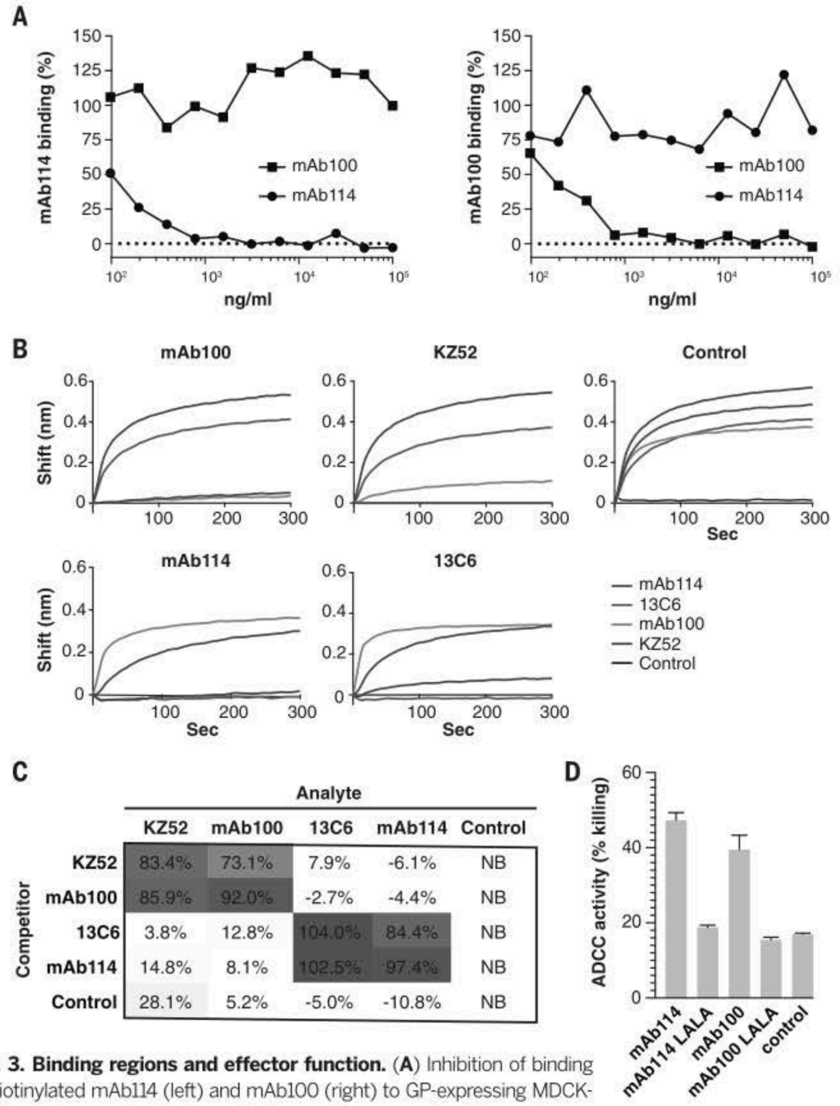

Fig. 3. Binding regions and effector function. (**A**) Inhibition of binding of biotinylated mAb114 (left) and mAb100 (right) to GP-expressing MDCK-SIAT cells by pre-incubation with increasing amounts of homologous or heterologous unlabeled antibodies. Shown is the percentage binding of biotinylated antibody (n = 1). (**B** and **C**) Biolayer interferometry competitive binding assay to soluble EBOV GP using mAb100, mAb114, KZ52, 13C6, and isotype negative control. Biosensors were preloaded with GP followed by the competitor and analyte antibodies as indicated. Analyte binding curves (B) and quantitated percent inhibition (C) are reported (n = 3, representative experiment shown). (**D**) Antibody-dependent cell-mediated cytotoxicity (ADCC) assay was determined for mAb100, mAb114 (n = 3, representative experiment shown), control antibody, or derivative antibodies with LALA mutations that abrogate Fc-mediated killing of HEK293T cells (n = 1), all at 31.6 ng/ml. ADCC activity is shown as mean ± SD.

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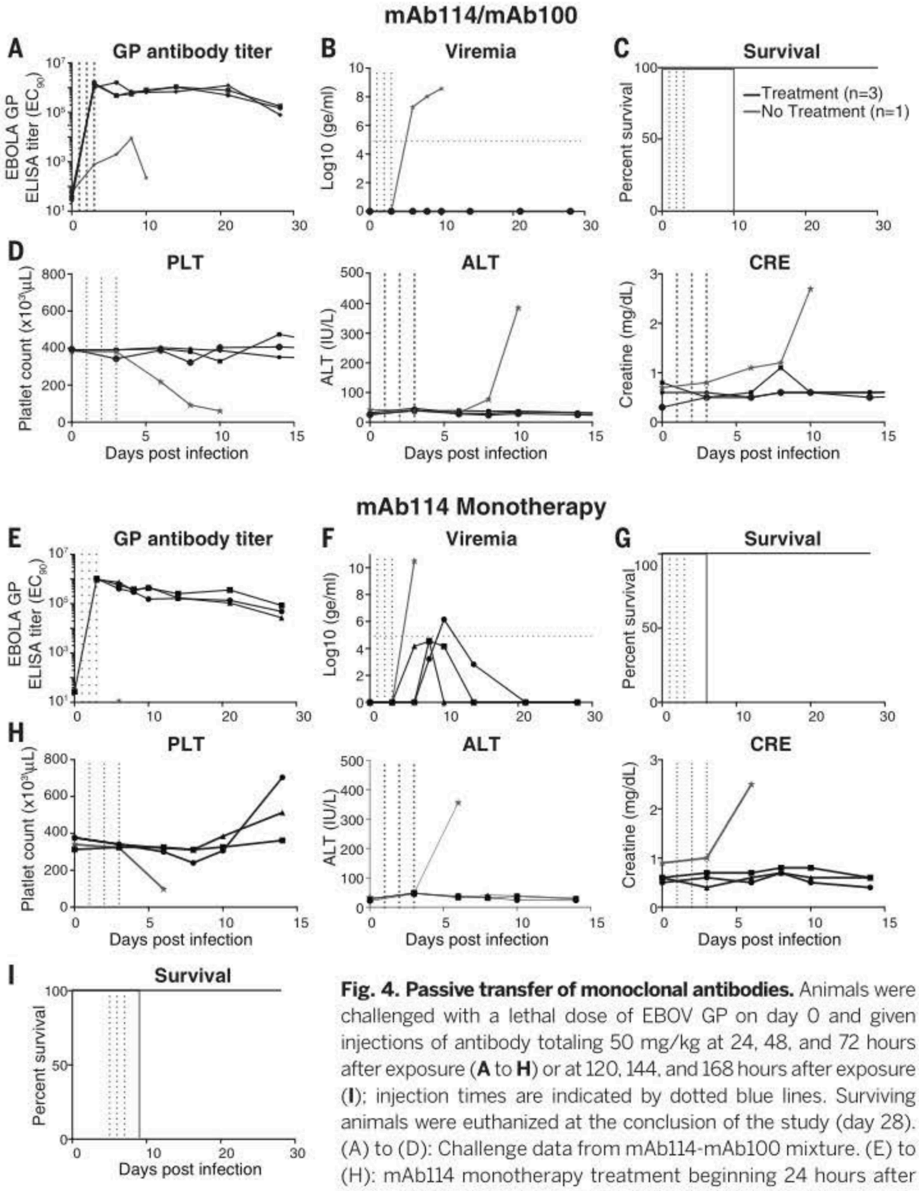

Fig. 4. Passive transfer of monoclonal antibodies. Animals were challenged with a lethal dose of EBOV GP on day 0 and given injections of antibody totaling 50 mg/kg at 24, 48, and 72 hours after exposure (**A** to **H**) or at 120, 144, and 168 hours after exposure (**I**); injection times are indicated by dotted blue lines. Surviving animals were euthanized at the conclusion of the study (day 28). (A) to (D): Challenge data from mAb114-mAb100 mixture. (E) to (H): mAb114 monotherapy treatment beginning 24 hours after exposure. (I): The same treatment beginning 120 hours after exposure. Black, treatment animals; red, untreated controls. (A) and (E): Ebola GP–specific ELISA titer (reciprocal EC_{90}). (B) and (F): Viremia in blood, as assessed by quantitative reverse transcription PCR (qRT-PCR). The dotted green lines denote the lower limit of quantitation of the qRT-PCR assay. (C), (G), and (I): Survival. (D) and (H): Selected hematologic and chemistry data. PLT, platelets; ALT, alanine transaminase; CRE, creatinine.

remained at levels less than 0.1% of the untreated control animal. Despite transient viremia, treated animals remained free of clinical and laboratory abnormalities (Fig. 4H and figs. S9 to S12).

Because a delay in treatment is a distinct possibility in an outbreak setting, we evaluated the therapeutic potential for mAb114 when treatment was delayed until 5 days after lethal EBOV challenge. All three animals in the treatment group survived, while the control animal succumbed to EVD on day 9. Moreover, animals in the treatment group remained symptom-free and protected against thrombocytopenia, transaminitis, and renal dysfunction (Fig. 4I and figs. S13 to S16).

mAb114 has several characteristics that may contribute to protection as a monotherapy when compared to KZ52 and 13C6, which were nonprotective in NHPs (*7*, *19*). First, although KZ52 and mAb114 potently neutralize EBOV in vitro, only mAb114 completely neutralizes input virus. Second, mAb114 does not require complement for neutralizing activity (Fig. 2B), in contrast to 13C6 (*6*). One hypothesis that might be derived from these observations is that protective monotherapy may require both potent binding and complete complement-independent neutralization. In addition, mAb114's specific mechanism of neutralization [which targets an essential step in virus entry (*20*)] and observed in vitro ADCC activity may contribute to mAb114's ability to protect against lethal EVD in macaques.

Our results show that antibodies as well as memory B cells specific to EBOV are maintained in a survivor more than a decade after infection. For the mAbs isolated from this survivor, a potential role for antibody-dependent killing is suggested by in vitro ADCC activity that in vivo may be mediated by multiple effector cells such as natural killer cells, macrophages, or neutrophils. In addition, these mAbs showed potent neutralizing activity against Ebola GP variants that have evolved over a 40-year period. Together, these data demonstrate the therapeutic potential of these mAbs as dual-combination therapy and/or monotherapy and contribute to understanding the mechanisms of antibody-mediated protection against Ebola virus disease.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/351/6279/1339/suppl/DC1
Materials and Methods
Figs. S1 to S16
References (*21*–*25*)

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Science

Protective monotherapy against lethal Ebola virus infection by a potently neutralizing antibody

Davide Corti, John Misasi, Sabue Mulangu, Daphne A. Stanley, Masaru Kanekiyo, Suzanne Wollen, Aurélie Ploquin, Nicole A. Doria-Rose, Ryan P. Staupe, Michael Bailey, Wei Shi, Misook Choe, Hadar Marcus, Emily A. Thompson, Alberto Cagigi, Chiara Silacci, Blanca Fernandez-Rodriguez, Laurent Perez, Federica Sallusto, Fabrizia Vanzetta, Gloria Agatic, Elisabetta Cameroni, Neville Kisalu, Ingelise Gordon, Julie E. Ledgerwood, John R. Mascola, Barney S. Graham, Jean-Jacques Muyembe-Tamfun, John C. Trefry, Antonio Lanzavecchia and Nancy J. Sullivan

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Antibodies block Ebola virus entry

The recent Ebola virus outbreak in West Africa illustrates the need for both an effective vaccine and therapies to treat infected individuals. Corti *et al.* isolated two monoclonal antibodies from a survivor of the 1995 Kikwit outbreak and demonstrated their therapeutic efficacy in Ebola virus–infected macaques. In fact, one antibody protected macaques when it was given up to 5 days after infection. Misasi *et al.* solved the crystal structures of fragments of the two antibodies bound to the Ebola virus glycoprotein (GP), which mediates viral cell entry. The two antibodies targeted different regions of GP, but in both cases blocked steps required for viral entry.

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EBOLA VIRUS

Structural and molecular basis for Ebola virus neutralization by protective human antibodies

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Ebola virus causes hemorrhagic fever with a high case fatality rate for which there is no approved therapy. Two human monoclonal antibodies, mAb100 and mAb114, in combination, protect nonhuman primates against all signs of Ebola virus disease, including viremia. Here, we demonstrate that mAb100 recognizes the base of the Ebola virus glycoprotein (GP) trimer, occludes access to the cathepsin-cleavage loop, and prevents the proteolytic cleavage of GP that is required for virus entry. We show that mAb114 interacts with the glycan cap and inner chalice of GP, remains associated after proteolytic removal of the glycan cap, and inhibits binding of cleaved GP to its receptor. These results define the basis of neutralization for two protective antibodies and may facilitate development of therapies and vaccines.

Ebola virus (EBOV) causes a rapidly fatal hemorrhagic fever for which there is currently no treatment (*1–3*). We recently isolated two monoclonal antibodies (mAb100 and mAb114) from a 1995 Kikwit Ebola survivor that potently neutralize multiple EBOV isolates spanning more than 40 years (*4*). When administered as a cocktail to rhesus macaques, these antibodies fully protected from clinical symptoms, viremia, and death. Furthermore, mAb114 monotherapy fully protected macaques from death and illness when given as late as 5 days after infection (*4*). In this study, we sought to identify the structural and molecular basis of neutralization for these protective antibodies.

The EBOV glycoprotein (GP) is a class I fusion protein comprising disulfide-linked subunits, GP1 and GP2, which associate to form a chalice-shaped trimer (*5–7*). The GP1 subunit binds to the EBOV receptor, Niemann-Pick C1 (NPC1), allowing GP2-mediated fusion of the viral and host-cell membranes (*5, 8–11*). The GP1 subunit contains a core domain and a "glycan cap," which are shielded by the heavily glycosylated mucin-like domain (MLD) (Fig. 1A). The MLD is dispensable for virus entry but is a target for host antibody responses (*6, 7, 12–16*). Using immunoprecipitation (IP), we found that mAb100 and mAb114 recognized GP ectodomains lacking the MLD ($GP_{\Delta Muc}$), suggesting that their epitopes reside elsewhere on GP (Fig. 1B) (*17*).

To identify the epitopes recognized by these antibodies, crystal structures of their antigen-binding fragments (Fab100 and Fab114) were determined individually to 2.0 Å and in a ternary complex with $GP_{\Delta Muc}$ to 6.7 Å (table S1 and fig. S1, A and B). The complex structure was solved by molecular replacement using the refined structures of the unbound Fabs and the previously solved EBOV $GP_{\Delta Muc}$ structure (*6*) as search models and was refined to an R_{work}/R_{free} of 26.0/34.3% (table S1). The crystal structure shows that Fab100 binds to the base of GP, parallel to the viral membrane, makes contacts with both GP1 and GP2, and cross-links two adjacent protomers (Fig. 1C and fig. S1C). In contrast, Fab114 binds within the GP chalice, perpendicular to the viral membrane, and makes contacts with both the glycan cap and the GP1 core (Fig. 1C and fig. S1C).

Because GP binds NPC1 in acidic late-endosomes and lysosomes (*8–12, 16*), we compared antibody binding to $GP_{\Delta Muc}$ at neutral and low pH using cryogenic electron microscopy (cryo-EM). The structures of the ternary complexes at pH 7.4 and 5.0 were calculated to a resolution of 9 Å by single-particle reconstruction (fig. S2). The ternary-complex crystal structure fit well as a rigid body into the cryo-EM densities (Fig. 2). Further rigid-body refinement of the Fabs and GP did not change the overall structure substantially, indicating that the crystal structure closely resembles the cryo-EM structure. Comparisons of the cryo-EM structures at pH 7.4 and 5.0 revealed highly similar structures (Fig. 2 and fig. S2C), suggesting that these antibodies would remain associated with GP during trafficking of EBOV to low-pH compartments. Analysis of the cryo-EM structure also revealed a bulky density near the Fab100 interface that would be consistent with an *N*-linked glycan at residue Asn563 of GP (*6, 7*) (fig. S3A). Enzymatic trimming of the glycans using endoglycosidase H (EndoH) did not appreciably alter Fab100 binding to GP, suggesting that *N*-linked glycans are not critical for Fab100 recognition (fig. S4, A to C).

Binding of Fab100 to the base of the GP trimer (Fig. 1C and fig. S1C) resembles that of KZ52, a prototypic neutralizing antibody that does not confer protection in macaques (*6, 18, 19*). However, Fab100 is rotated about the trimeric axis by ~60° with respect to KZ52 (fig. S3B). This rotation enables Fab100 to contact GP1 and GP2 of one protomer, as well as the internal fusion loop (IFL) of the neighboring protomer, whereas KZ52 contacts only a single protomer (fig. S3C). Fab100 is also in close proximity to the β13-β14 loop (Fig. 3A) (residues 190 to 213), which is disordered in the previous structure (*6*). Biochemical studies have shown that EBOV entry requires cleavage of this loop by cathepsins L and B (*11, 12, 20–23*), which releases the glycan cap and MLD, exposing the receptor-binding domain (RBD) within the GP1 core (*8–12, 20, 21*). Interestingly, the cryo-EM structure revealed additional electron density corresponding to portions of the β13-β14 loop in close proximity to the Fab100 light chain (Fig. 3A). The observed density would be expected to accommodate residues 190 to 197 and 209 to 213 and may accommodate additional residues depending on the conformation of the β13-β14 loop, which is difficult to determine given the weak electron density of this region. We therefore hypothesized that Fab100 would sterically block proteolysis by cathepsins. To test this, we used cathepsin L (Cat L) to digest $GP_{\Delta Muc}$ that was pretreated with mAb114, mAb100, KZ52, or control mAb. The amount of cleaved GP1 ($GP1_{20k}$) was similar in the control and mAb114 reactions (Fig. 3B). Consistent with previous reports, KZ52 delayed the appearance of $GP1_{20k}$ (*24*). For mAb100, the primary product was an intermediate form (GP1i) with only trace amounts of $GP1_{20k}$, indicating that mAb100 significantly reduced GP1 cleavage (Fig. 3B). Similarly, cleavage of GP1 by thermolysin, which mimics cathepsin B (Cat B) (*8, 11, 20, 23*), was inhibited by mAb100 (fig. S4D). These data suggest that mAb100 neutralizes EBOV by sterically blocking cathepsin cleavage of the β13-β14 loop.

We next determined the affinity of Fab100 and KZ52 for $GP_{\Delta Muc}$ at neutral and low pH. The affinity of Fab100 was ~5 and ~10 times stronger than KZ52 at pH 7.4 and 5.3, respectively (Fig. 3C and fig. S4A), suggesting that mAb100 remains tightly associated with GP in low-pH compartments. Notably, the affinity of KZ52 for thermolysin-cleaved GP (GP_{THL}) at

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pH 5.3 was decreased by a factor of more than 1000 as compared with uncleaved GP. For Fab100, a modestly reduced affinity for GP_{THL} was driven primarily by a faster dissociation rate (Fig. 3C and fig. S4A). However, the dissociation rate constant (k_{off}) of the bivalent mAb100 immunoglobulin G (IgG) was similar between uncleaved and GP_{THL} (fig. S5), suggesting that mAb100 remains bound even after proteolytic cleavage at low pH. Due to the quaternary nature of the mAb100 epitope, which includes the internal fusion loop, mAb100 may also prevent conformational rearrangements of GP that occur downstream of proteolytic cleavage. Antibodies with quaternary epitopes have been recently identified that potently neutralize other viruses, suggesting that this mode of binding represents a powerful immunological solution to viral entry (*25–27*).

Unlike mAb100, mAb114 recognizes an epitope spanning both the glycan cap and the GP1 core (Figs. 4A and 1C). Most contacts appear to be made between the CDR H3 and L3 of Fab114 and the loop connecting β8 and β9 of the GP1 core (*6*). The importance of this region for mAb114 binding was confirmed by CLIPS (chemical linkage of peptides onto scaffolds) conformational-epitope mapping (*28*) (fig. S6A). Because the contacts within the GP1 core remain after cathepsin cleavage (fig. S7A), we investigated mAb114 binding to GP_{THL} using IP and found that GP_{THL} was recognized similarly to GP and $GP_{\Delta Muc}$ (Fig. 4B). Furthermore, negative-stain EM showed that the binding orientation of Fab114 to GP_{THL} was similar to $GP_{\Delta Muc}$ (Fig. 4C), demonstrating that the glycan cap is dispensable for mAb114–GP interaction.

Because EBOV particles transit from neutral to low-pH compartments (*12, 16, 20*), we measured binding kinetics of Fab114 to $GP_{\Delta Muc}$ at pH 7.4 and 5.3 and observed similar subnanomolar affinities (Fig. 4D and fig. S6B). When compared with antibody 13C6, which competes with mAb114 for binding to GP (*4*) but is not protective in macaques (*15, 29*), Fab114 has a significantly slower k_{off}, leading to an affinity that is tighter by factors of more than 250 and 40 at pH 7.4 and 5.3, respectively (Fig. 4D and fig. S6B). Importantly, 13C6 only makes contact with the glycan cap (*30*), whereas mAb114 bound GP after glycan cap removal (Fig. 4, B and C). The affinity of Fab114 for GP_{THL} at pH 5.3 remained high [equilibrium dissociation constant (K_D) of 8.0 nM], consistent with our structural data showing that Fab114 primarily contacts residues within the GP1 core.

After cathepsin cleavage of GP1, a hydrophobic pocket is exposed on the GP1 core that is formed primarily by α1 and β4 and bordered by charged residues in β7, β8, and β9. These regions have been proposed to be the RBD of EBOV GP (*6, 23, 31–36*). Our data suggested that the interaction of mAb114 with residues in β7 to β9 might block NPC1 access to this pocket. To investigate this, we performed a competition assay with mAb114, GP_{THL}, and NPC1 domain C (NPC1-dC)—the domain responsible for engaging cleaved GP and mediating virus entry (*8–10*). Using biolayer interferometry, we found that when mAb114 was bound to GP_{THL}, NPC1-dC was unable to bind (Fig. 4E and fig. S6C). Similar results were obtained using IP (fig. S6D). These findings are consistent with the observation that both Fab114 and NPC1-dC have similar affinities for GP_{THL} (Fig. 4D and fig. S6B) and indicate that mAb114 neutralizes EBOV infection by preventing binding of cathepsin-cleaved GP to its receptor NPC1.

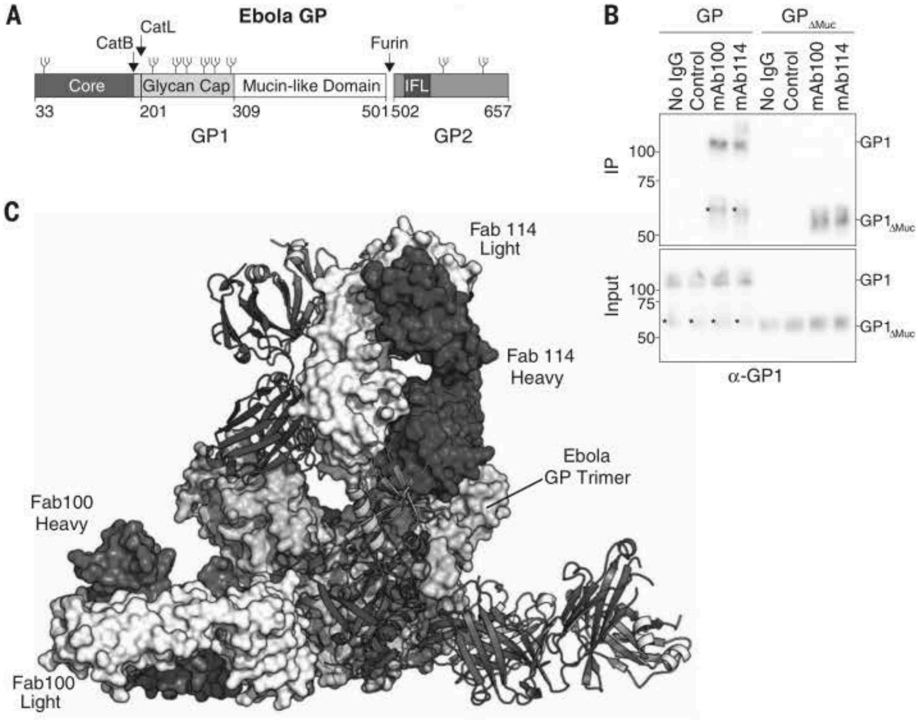

Fig. 1. Binding requirements and structure of antibodies in complex with GP. (**A**) Schematic representation of GP monomer, colored by domain. GP1 core region (33 to 190) is colored blue, GP1 glycan cap is colored yellow (201 to 308), and the mucin-like domain is uncolored (309 to 501). The GP2 IFL is colored red, and the remainder of GP2 is colored orange. Glycans are shown as branched lines, and proteolytic cleavage sites are labeled with arrows. Disulfide bonds within and between GP1 and GP2 are omitted for clarity. (**B**) IP of soluble GP ectodomain containing or lacking the mucin-like domain ($GP_{\Delta Muc}$) by mAb100, mAb114, or isotype control. Binding and input were analyzed using immunoblotting for GP1. *GP1 degradation product present only in mucin-containing GP. $n = 3$ replicates; representative image shown. (**C**) Crystal structure of $GP_{\Delta Muc}$ in complex with Fab100 and Fab114. Fab100 is shown in purple (heavy chain) and white (light chain). Fab114 is shown in pink (heavy chain) and white (light chain). Molecular surfaces of two $GP_{\Delta Muc}$ protomers are colored in green and beige, whereas the third is shown as a ribbon representation and colored according to the schematic in (A).

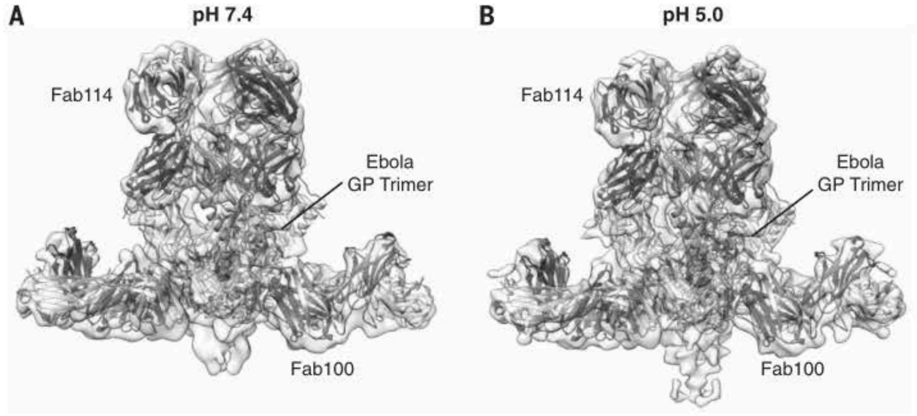

Fig. 2. Cryo-EM of $GP_{\Delta Muc}$-Fab100-Fab114 complex. Cryo-EM was performed on the ternary complex of $GP_{\Delta Muc}$ with Fab100 and Fab114 at (**A**) pH 7.4 and (**B**) pH 5.0. Shown are the superimpositions of the crystal structure (ribbon) into the cryo-EM density maps at their respective pH.

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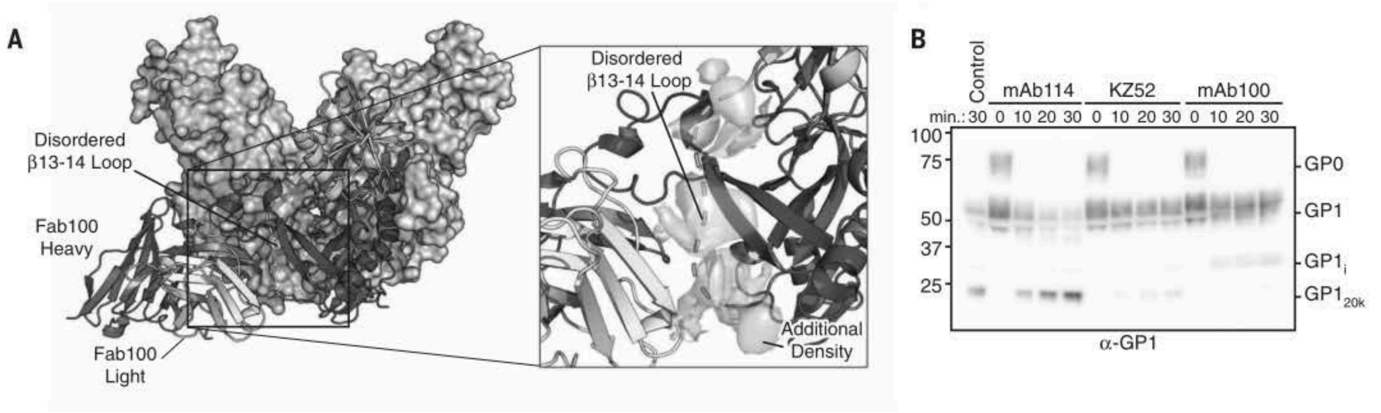
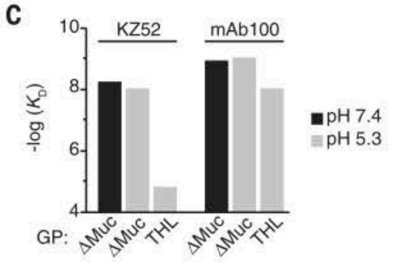


Fig. 3. mAb100 inhibits cathepsin cleavage of GP. (**A**) Fab100 binding occludes access to the β13-β14 loop of GP1. Protomers and Fab100 heavy and light chains are colored and oriented as in Fig. 1C. The variable domain of one Fab100 is shown in a ribbon representation, and all other Fabs are removed for clarity. Inset shows a zoomed view of the Fab100-GP interface with the difference map generated by masking out the cryo-EM densities of the fitted crystal structures shown as a gray transparent surface. The β13-β14 loop is shown as a dashed yellow line connecting the GP1 core (blue) and the glycan cap (yellow). (**B**) $GP_{\Delta Muc}$ was incubated with the indicated antibodies followed by cleavage at pH 5.5 by Cat L at 37°C. Samples were removed at 5-min intervals and analyzed by immunoblot for GP1. n = 3 replicates; representative image shown. (**C**) Binding kinetics, as determined by biolayer interferometry, of $GP_{\Delta Muc}$ or GP_{THL} (THL) with Fab100 or KZ52 at the indicated pH. K_Ds are plotted on a negative log scale. n = 2 replicates; representative experiment shown.



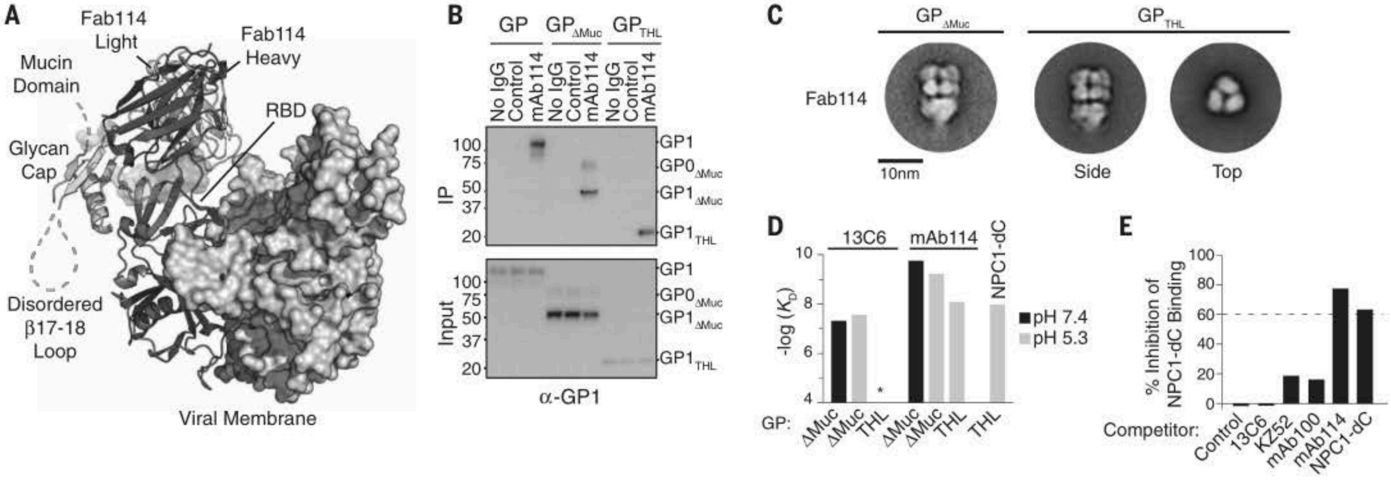


Fig. 4. mAb114 blocks binding of NPC1 to the GP1 core. (**A**) Fab114 binds to regions in the glycan cap and core of GP1. Protomers are colored as in Fig. 1C and viewed with a 100° rotation about the trimeric axis with respect to the orientation in Fig. 1C. The variable domain of a single Fab114 is shown in ribbons, and all other Fabs have been removed for clarity. GP residues predicted to make contact with Fab114 are shown as transparent surfaces. (**B**) Immunoprecipitation of $GP_{\Delta Muc}$ and GP_{THL} by the indicated antibodies. Samples were analyzed by immunoblot for GP1. n = 3 replicates; representative image shown. (**C**) Class averages of single particles from negative-stain electron micrographs of Fab114 in complex with $GP_{\Delta Muc}$ and GP_{THL}. (**D**) Binding kinetics, as determined by biolayer interferometry, of $GP_{\Delta Muc}$ or GP_{THL} with Fab114, 13C6, or NPC1-dC at the indicated pH. K_Ds are plotted on a negative log scale. *No binding. n = 2 replicates; representative experiment shown. (**E**) Inhibition of NPC1-dC binding to GP_{THL} by competitor proteins (NPC1-dC) or antibodies (mAb100, mAb114, 13C6, KZ52, or isotype control) was determined by biolayer interferometry. Dashed line represents 60% inhibition of binding. n = 3 replicates; representative experiment shown.

Despite being in the same competition group as mAb114 (*4*), antibody 13C6 fails to neutralize EBOV due to its inability to remain bound to GP after cathepsin cleavage. Conversely, the Marburg GP-antibody MR78 recognizes an analogous receptor-binding domain on Marburg GP and also binds to cleaved EBOV GP, yet fails to neutralize EBOV due to its inability to recognize the native trimer (uncleaved GP) (*34*, *35*). The failure of MR78 to bind native EBOV GP led to the hypothesis that NPC1-blocking antibodies might not be elicited during Ebola virus infection (*34*). Strikingly, mAb114 overcomes these structural constraints by binding to the center of the GP1 chalice with a near-vertical angle of approach (85° with respect to the viral membrane) that allows access to the GP1 core. The recently published crystal structure of NPC1-dC bound to GP_{THL} revealed that in addition to making contacts with the hydrophobic pocket exposed by removal of the glycan cap and MLD, NPC1 also contacts the surrounding charged region in β7 to β9 that is bound by mAb114 (fig. S7B) (*36*). Taken together, these data reveal a key site of vulnerability in the EBOV GP targeted by mAb114 and demonstrate that this class of antibodies can be elicited by natural infection.

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The experiments herein reveal that mAb100 and mAb114 mediate virus neutralization by targeting independent essential steps in EBOV entry: exposure of the RBD by protease cleavage and receptor binding. Because these steps are required for the entry of all members of the *Filoviridae* family (*8*, *11*), our studies identify vulnerabilities targeted by the host immune system that could potentially be exploited in vaccine and therapeutic development.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/351/6279/1343/suppl/DC1
Materials and Methods
Figs. S1 to S7
Table S1
References (*37*–*55*)

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Science

Structural and molecular basis for Ebola virus neutralization by protective human antibodies

John Misasi, Morgan S. A. Gilman, Masaru Kanekiyo, Miao Gui, Alberto Cagigi, Sabue Mulangu, Davide Corti, Julie E. Ledgerwood, Antonio Lanzavecchia, James Cunningham, Jean Jacques Muyembe-Tamfun, Ulrich Baxa, Barney S. Graham, Ye Xiang, Nancy J. Sullivan and Jason S. McLellan

Science **351** (6279), 1343-1346.
DOI: 10.1126/science.aad6117originally published online February 25, 2016

Antibodies block Ebola virus entry

The recent Ebola virus outbreak in West Africa illustrates the need for both an effective vaccine and therapies to treat infected individuals. Corti *et al.* isolated two monoclonal antibodies from a survivor of the 1995 Kikwit outbreak and demonstrated their therapeutic efficacy in Ebola virus–infected macaques. In fact, one antibody protected macaques when it was given up to 5 days after infection. Misasi *et al.* solved the crystal structures of fragments of the two antibodies bound to the Ebola virus glycoprotein (GP), which mediates viral cell entry. The two antibodies targeted different regions of GP, but in both cases blocked steps required for viral entry.

Science, this issue pp. 1339 & 1343

ARTICLE TOOLS http://science.sciencemag.org/content/351/6279/1343

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AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Sat, 12 May 2018 19:38:32 +0000
To: Bluestone, Jeffrey (Private)
Cc: (b)(6) Emily (NIH/NIAID) [E];Marston, Hilary (NIH/NIAID) (b)(6)
Subject: FW: Ebola and MAS
Attachments: Anakinra for Ebola proposal 2018.pdf, Interleukin_1_Receptor_Blockade_Is_Associated_With.5.pdf, Ebola biomarkers 2014.pdf, J Rheumatol-EBOLA editorial 2015[2].pdf, Ebola MAS Frontiers in Medicine 2015[2].pdf, Ebola and ferritin[2].pdf

Jeff/Randy:

Thanks for the note and for sending the attached material. My immediate reaction from the proposal and the associated literature is that IL-1 blockade is an intriguing hypothesis well worth exploration in the non-human primate (NHP) lethal challenge model compared to other available immunotherapeutics (e.g. mAb cocktails such as ZMapp) assuming Anikinra has a rhesus equivalent that could be used, However, this concept is really not ready for potential use in human cases in the current or future outbreaks until such animal testing has been undertaken and proven safe. Having said that, I am copying Emily Erbelding, Director of NIAID's Division of Microbiology and Infectious Diseases (DMID) and asking her by this e-mail to reach out to you (Randy) to open a dialogue to determine if DMID could assist you in further development of this concept.

Best regards,
Tony

From: Bluestone, Jeffrey (Private) (b)(6)
Sent: Friday, May 11, 2018 4:14 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: Ebola and MAS

Tony, you may remember that the last time there was a big Ebola outbreak, I forwarded a suggestion made by an former grad student/post-doc of mine, Randy Cron, who is currently Chair of Pediatric Rheumatology at UAB. He had hypothesized that much of the morbidity of Ebola was due to an MAS syndrome and suggested that it would be worth trying Anakinra (the il-1 antagonist) in a few patients. I think at the time, you had suggested he continue to study and perhaps put in a grant. Since that time he and others have continued to study the similarities between Ebola morbidity and MAS. He remains convinced that there would be great value. I met with Hugh yesterday and he told me how involved you are in these outbreaks and the commitment you having including travel to Africa and direct involvement in the treatment of patients coming back to US. I also know that Cliff Lane is very involved. I am attaching a letter from Randy and some papers with the hope you or your colleagues involved in this new outbreak might open a dialogue and see if such a treatment is worth trying.

AMERICAN OVERSIGHT

Jeff

==========

Jeffrey A. Bluestone, Ph.D.
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AMERICAN OVERSIGHT

UAB MEDICINE
PEDIATRIC RHEUMATOLOGY

Children's of Alabama

May 11, 2018

Anthony Fauci, MD
Director, NIAID

Dear Dr. Fauci:

As you are aware, Ebola is a rapidly fatal viral infection that has returned to W. Africa in recent days [https://www.cnn.com/2018/05/10/health/drc-confirms-first-ebola-death/index.html]. At present, there are no known effective therapies, and the toll from this current Ebola outbreak may potentially reach millions of people.

A study from the Centers for Disease Control (CDC) detailing the 2000-2001 Ebola outbreak noted that elevated serum ferritin levels were strongly associated with both fatal outcomes and hemorrhagic features of disease (1). Depending on the cut-off value, elevated serum ferritin can be both a sensitive and specific marker of cytokine storms syndromes, such as macrophage activation syndrome (MAS) and hemophagocytic lymphohistiocytosis (HLH) (2). HLH/MAS is a frequently fatal complication of infectious, oncologic, and rheumatic conditions that results from a pro-inflammatory cytokine storm (3). The clinical, laboratory, and pathologic features of HLH/MAS are strikingly similar to Ebola and other forms of hemorrhagic fever, including high fever, elevated serum ferritin values, liver dysfunction, pancytopenia, hemophagocytosis, central nervous system dysfunction, hemorrhages, etc. (3).

HLH and hemophagocytosis have already been associated with other hemorrhagic fever viruses, including Crimean-Congo (4) and Dengue (5). Indeed, elevated serum ferritin levels have been reported in Dengue (6) and associated with the severity of HLH manifestations in Crimean-Congo fever (7). It is, therefore, not much of a stretch to consider Ebola hemorrhagic fever as a likely trigger of HLH/MAS.

Familial HLH with homozygous defects in lymphocyte cytolytic pathway genes is uniformly fatal unless treated with an aggressive etoposide-based protocol (HLH-2004) followed by bone marrow transplantation (3). Secondary HLH or MAS presenting beyond infancy is often treated with immunosuppressive therapy such as high dose corticosteroids and cyclosporine A (8). Indeed, immunosuppression has successfully treated Dengue (9). Recently, targeted cytokine inhibition has been shown to successfully treat HLH/MAS (10). In particular, blockade with the drug anakinra, a recombinant human IL-1 receptor antagonist (IL-1Ra), has been successfully used to treat refractory cases of MAS (11). Moreover, anakinra was safe and doubled the survival of patients with sepsis (12). Experimentally, IL-1Ra deficient mice were shown to have enhanced susceptibility to Ebola virus infection (13). Finally, we are currently conducting a randomized, double-blinded placebo controlled trial of anakinra in treating non-oncologic associated MAS [NCT02780583].

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The benefits of anakinra specifically are the following:

1) It is quick acting. Within 24-72 hours, a clinical and laboratory response will be evident (14).
2) It is remarkably safe in humans (>10,000 received) as a recombinant human protein (15).
3) It has a very short half-life (4-6 hours), so it is rapidly cleared should safety concerns arise (15).
4) It is easily administered intravenously (IV), or subcutaneously when IV access is limited (16).
5) It has a large therapeutic window with safe dosing in humans ranging from 1 to 48 mg/kg/day (17).
6) It binds both interleukin 1 alpha and beta (IL-1α and IL-1β) (15).

For the current outbreak of Ebola in W. Africa, we, therefore, propose to administer anakinra (100 mg IV/SC q 6 hours) (18) early in the course of disease in individuals with a clinical diagnosis of Ebola hemorrhagic fever. The ability to administer anakinra subcutaneously bypasses the necessity of IV access which proved difficult in the last large Ebola outbreak in W. Africa. We suggest monitoring fever, overall clinical well-being, and any or all of the following labs if available: AST, ALT, D-dimers, platelet count, CRP, and ferritin. Responses should be evident within 24-72 hours of administration with very little risk, infectious or otherwise, but a large potential rapid benefit to the patient. Anakinra serves as a powerful anti-pyretic if nothing else. As we and others have suggested previously (19, 20), we hope you consider this relatively simple to administer, quick acting, and remarkably safe agent in treating those suffering from Ebola.

Happy to discuss by phone at your convenience.

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Interleukin-1 Receptor Blockade Is Associated With Reduced Mortality in Sepsis Patients With Features of Macrophage Activation Syndrome: Reanalysis of a Prior Phase III Trial*

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Objective: To determine the efficacy of anakinra (recombinant interleukin-1 receptor antagonist) in improving 28-day survival in sepsis patients with features of macrophage activation syndrome. Despite equivocal results in sepsis trials, anakinra is effective in treating macrophage activation syndrome, a similar entity with fever, disseminated intravascular coagulation, hepatobiliary dysfunction, cytopenias, and hyperferritinemia. Hence, sepsis patients with macrophage activation syndrome features may benefit from interleukin-1 receptor blockade.

Design: Reanalysis of deidentified data from the phase III randomized interleukin-1 receptor antagonist trial in severe sepsis.

Setting: Multicenter study recruiting through 91 centers from 11 countries in Europe and North America.

Patients: Sepsis patients with multiorgan dysfunction syndrome and/or shock (original study) were regrouped based on the presence or the absence of concurrent hepatobiliary dysfunction and disseminated intravascular coagulation as features of macrophage activation syndrome. The non–hepatobiliary dysfunction/disseminated intravascular coagulation group included patients with only hepatobiliary dysfunction, only disseminated intravascular coagulation, or neither.

Intervention: Treatment with anakinra or placebo.

Measurements and Main Results: Main outcome was 28–day mortality. Descriptive and comparative statistics were performed. Data were available for 763 adults from the original study cohort, randomized to receive either anakinra or placebo. Concurrent hepatobiliary dysfunction/disseminated intravascular coagulation was noted in 43 patients (5.6% of total; 18–75 years old; 47% women). The 28-day survival was similar in both anakinra and placebo-treated non–hepatobiliary dysfunction/disseminated intravascular coagulation patients (71.4% vs 70.8%; $p = 0.88$). Treatment with anakinra was associated with significant improvement in the 28-day survival rate in hepatobiliary dysfunction/disseminated intravascular coagulation patients (65.4% anakinra vs 35.3% placebo), with hazard ratio for death 0.28 (0.11–0.71; $p = 0.0071$) for the treatment group in Cox regression.

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Conclusions: In this subgroup analysis, interleukin-1 receptor blockade was associated with significant improvement in survival of patients with sepsis and concurrent hepatobiliary dysfunction/disseminated intravascular coagulation. A prospective randomized trial using features of macrophage activation syndrome for mortality risk stratification should be undertaken to confirm the role of interleukin-1 blockage. (*Crit Care Med* 2016; 44:275–281)

Key Words: anakinra; disseminated intravascular coagulation; hepatobiliary dysfunction; interleukin-1 receptor antagonist; macrophage activation syndrome; multiorgan dysfunction syndrome; sepsis mortality

"Sepsis" constitutes various degrees of systemic inflammation in the context of microbial infection (1), with production of proinflammatory cytokines including interleukin-1 (IL-1), IL-6, IL-18, and tumor necrosis factor (2, 3). These cytokines may contribute to development of sepsis-induced multiorgan dysfunction syndrome (MODS) and/or hypoperfusion, identified as severe sepsis and septic shock, respectively, based on International Sepsis Definitions Conference guidelines (1).

Following the discovery of IL-1 as human leukocytic pyrogen in 1977 (4), IL-1 receptor antagonist (IL-1Ra) was purified in 1981 (5) and was demonstrated to have an important role in regulating systemic inflammatory responses (3–6). IL-1Ra (3, 7) is a 25 kD naturally occurring plasma protein that binds to the type 1 IL-1 receptor, inhibiting both IL-1α and IL-1β signal transduction (7). Anakinra is a 17-kD recombinant, nonglycosylated human IL-1Ra. Prior to its approval to treat other inflammatory diseases (8), anakinra was investigated in a series of clinical trials for treatment of patients with sepsis and severe sepsis (9–12). An initial phase I study was conducted with IV doses as high as 10 mg/kg/hr for 72 hours (9), which established the safety and tolerability of anakinra at high doses. A subsequent phase II open-label clinical trial demonstrated dose-related efficacy in sepsis syndrome and septic shock without treatment-related adverse outcomes (10). In an ensuing phase III randomized double-blind trial comparing 1 or 2 mg/kg/hr with placebo, anakinra failed to meet the primary endpoint of improved 28-day survival (11), but did improve survival in patients with MODS, a predicted mortality probability of greater than 24% at study entry, or septic shock (11). On the basis of these results, a subsequent confirmatory phase III trial was undertaken in patients with severe sepsis or septic shock (12). This latter study was discontinued following an interim analysis, in which no survival benefits could be demonstrated (12). This, and the other clinical trials of anakinra in sepsis, did not reveal either increased mortality rate or serious/nonserious adverse reactions in anakinra over placebo groups (9–12).

In contrast to its effects in sepsis, anakinra, either alone or in combination with other treatments, has been reported to be effective in the management of macrophage activation syndrome (MAS), previously termed "secondary or reactive hemophagocytic lymphohistiocytosis" (13). Clinically, MAS presents as a fulminant cytokine storm and often fatal cause of MODS (13–17), associated with pancytopenia, tissue hemophagocytosis, liver dysfunction, coagulopathy, and/or CNS dysfunction (18–22), occurring as a complication of infection, malignancies, and autoimmune disorders (13, 15). The excessive inflammatory response in MAS is reflected in the amplified transcription of ferritin, and tissue-bound and soluble CD163 (23–25), both of which have also been demonstrated in severe sepsis (26–31).

Given the clinical and cytokine similarities of MAS and severe sepsis, together with the observed favorable response of MAS to anakinra, the possibility that severe sepsis patients with features of MAS may respond to IL-1R blockade merits consideration. This study revisits the data from the confirmatory phase III randomized clinical trial of IL-1Ra (anakinra) in severe sepsis (12), reanalyzing the data to investigate the response to anakinra in patients with features of MAS (hepatobiliary dysfunction [HBD] and disseminated intravascular coagulation [DIC]) (18–22).

PATIENTS AND METHODS

Data Source

The data from the previously published original confirmatory phase III placebo-controlled trial of IL-1Ra in severe sepsis (12) were reanalyzed. In this trial, 906 patients with severe sepsis and septic shock were recruited from 91 centers in 11 countries across Europe and North America, randomly assigned to either treatment or placebo (1:1 ratio). The primary endpoint was defined as 28-day survival; end-organ dysfunction and/or failure were determined by consensus of a steering committee according to the guidelines in the antecedent phase III clinical trial (11) with additional modifications (12). The original study was stopped at the second interim analysis for futility. At the time of study termination, 58 patients had completed the 3-day infusion (drug or placebo) and were in various stages of 28-day follow-up. A group of 143 patients was in various stages of screening and infusion, without completing the full infusion course. This group was dropped from the study, and their data were not available for the current analysis.

Regulatory Process

Each participating center obtained institutional review board approval, and all subjects gave written informed consent to participate in the original trial. The dataset included demographic, clinical, and outcome data for subjects in the study, each identified by a study-specific number. None of the personal identifiers were disclosed in the dataset that was used for this study.

Variables

The available data for each subject used for this study included age, gender, presence or absence of shock, DIC, HBD, or acute kidney injury (AKI), survival at day 28, predicted mortality, and treatment. Definitions of DIC and organ dysfunction (HBD, shock, acute respiratory distress syndrome [ARDS], and AKI) were as described previously in the original open-label trial (11) (**Table 1**). The definitions of shock and DIC were amended in the confirmatory phase III clinical trial (12). Twenty-eight–day

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survival was the primary endpoint of this study. The Acute Physiology and Chronic Health Evaluation (APACHE) II score was used to calculate predicted death, which strongly correlated with APACHE II (11, 12). The APACHE II score and the specifics of infecting organisms were not available as part of the dataset. All of the subjects with missing data for treatment, 28-day outcome, or both DIC and HBD domains were excluded, as these were the main variables of interest in this study.

Disease and Nondisease Groups

In keeping with the objective of the study, features of MAS were defined as the presence of both HBD and DIC in this severe sepsis cohort. This choice of definition was guided by the clinical characteristics of MAS as determined in major studies (18–22), and each patient was classified into one of the two main groups of interest:

- HBD/DIC group: patients whose clinical presentation met the definitions of both HBD and DIC and
- non-HBD/DIC group: patients who did not have both HBD and DIC and included patients with only HBD, only DIC, or neither.

Treatment Versus Placebo

The original study was a randomized, placebo-controlled study. Patients in each group had, therefore, been randomly assigned at a 1:1 ratio to receive either placebo or anakinra, administered IV at 2.0 mg/kg/hr for 72 hours continuously.

Statistical Analysis

Statistical analysis was performed to compare demographic, clinical, and outcome variables between four subgroups comprised HBD/DIC and non-HBD/DIC patients who received anakinra or placebo. Descriptive statistics was performed for the variables, using mean and SD for continuous variables (age and predicted death), or proportion and percent for binomial variables. Comparative statistics included *t* tests for continuous variables, as well as Fisher Exact test and chi -square test for binomial variables. Kaplan-Meier survival estimates were also compared between the drug and control groups who were HBD/DIC positive, HBD/DIC negative, or HBD/DIC equivocal. The possibility of postrandomization imbalances was investigated by comparing pretreatment variables in drug versus control groups, using between-group *t* test or chi-square test. Drug effects were

TABLE 1. Variable Definitions Used in the Original Study by Opal et al (12)

Variable	Definition
Shock	Either of the following: • Systolic blood pressure ≤ 90 mm Hg, mean arterial pressure ≤ 70 mm Hg, decrease in systolic blood pressure ≥ 40 mm Hg • Persistent need for vasopressor agents to maintain blood pressure (excluding dopamine at dosages < 5.0 μg/kg/min) despite adequate fluid resuscitation
Disseminated intravascular coagulation	Both criteria No anticoagulation • PT or partial thromboplastin time ≥ 1.2 × normal value • Platelet count ≤ 100×10^9/L or ≥ 50% decrease in platelet count With therapeutic doses of warfarin or heparin • Abnormal platelet counts (above) and • Elevated fibrin split products or the D-dimer ≥ 0.5 mg/L
Acute kidney injury	Any of the following • Serum creatinine concentration ≥ 177 mmol/L (≥ 2.0 mg/dL) • Need for dialysis • Pre-existing renal dysfunction: ≥ 177 mmol/L (≥ 2.0 mg/dL) increase in the serum creatinine concentration
Hepatobiliary dysfunction	At least two of the following • Total serum bilirubin concentration ≥ 43 mmol/L (≥ 2.5 mg/dL) • Serum concentration of aspartate aminotransferase or alanine aminotransferase ≥ 2 × upper limit normal • PT ≥ 1.5 × normal value
Acute respiratory distress syndrome	According to criteria of Murray et al (47)

PT = prothrombin time.

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examined while controlling for potential confounders using logistic regression, as well as Cox proportional hazards models. A logistic regression model predicting 28-day survival was examined in the HBD/DIC subgroup to determine the association of drug with outcome, after controlling for potentially confounding covariates. To test whether the drug effect differed significantly across patients who were HBD/DIC positive, HBD/DIC negative, or HBD/DIC equivocal (defined as having either HBD positive or DIC positive but not both), we also tested a logistic regression model in the full sample using a drug × diagnosis interaction term in the model. To rule out the possibility that multicolinearity might have affected our multivariate models, we tested for this by examining variance inflation in weighted regression. SAS version 9.3 (Cary, NC) was used for all analyses. A nominal p value of less than 0.05 between groups was considered statistically significant.

RESULTS

Subject Group

Deidentified data were available on 763 study patients who had completed the study. All of these study patients were included in the analysis. The necessary variables for the current subgroup investigation were available in all of the patients. Of these, 43 patients (46.6% women) demonstrated both HBD and DIC; 26 (60.4%) were treated with anakinra (17 placebo). The remaining 720 subjects (40.7% women) were similarly distributed in anakinra or placebo groups: 484 subjects (67.2%) on anakinra, 236 subjects receiving placebo (**Table 2**). Despite the planned 1:1 randomization of the patients in the original trial, the observed ratio of anakinra to placebo group in the studied patient cohort was 2:1. A similar distribution is observed in the HBD/DIC subgroup. This has been confirmed with the investigators of the study to be merely a chance finding, likely related to early termination of the study.

Patient Characteristics in HBD/DIC and Non-HBD/DIC Groups

Clinical and outcomes characteristics of the patients are reported in the original study publication (12). The descriptive details of the subgroups reported herein are outlined in Table 2. In comparative analysis, the HBD/DIC group subjects were significantly younger than their non-HBD/DIC counterparts ($p = 0.025$) with significantly higher predicted mortality scores at study entry ($p = 0.0001$). The HBD/DIC patients were less likely to be alive at 28 days (53.5% vs 70.0%; $p = 0.027$). Average survival duration in HBD/DIC patients was 16.5 days, significantly shorter than the 21.7 days in non-HBD/DIC patients ($p = 0.0004$). ARDS was noted in similar proportions in both groups, but AKI and shock were both more frequent among the HBD/DIC patients ($p = 0.0001$ for AKI; $p = 0.0089$ for shock).

Mortality and Treatment With Anakinra

The outcome of death related to anakinra versus placebo treatment was determined over the course of the study observation period for HBD/DIC and non-HBD/DIC patients. As shown in **Figure 1**, 28-day mortality was equivalent for the non-HBD/DIC patients who received anakinra (29.7%) or placebo (30.5%; $p = 0.999$). However, the 28-day mortality in HBD/DIC patients who received anakinra was significantly lower (34.6%) than was noted for HBD/DIC patients who received placebo (64.7%; $p = 0.0006$), corresponding to a 47% reduction in mortality associated with anakinra.

Confirmatory Analysis

Given the post hoc nature of this investigation, confirmatory statistical evaluations were performed to identify any postrandomization imbalances or confounders. We confirmed that the predicted death probability score was normally distributed with a mean 0.36 ± 0.21 and a median = 0.32, in the full sample. Probability of death was well balanced between treatment and placebo groups (mean predicted death: 0.358 ± 0.21 in the treated group [$n = 510$] and 0.354 ± 0.21 in the placebo group [$n = 253$]; $p = 0.81$). The risk of death between HBD/DIC patients who received anakinra treatment (mean, 0.57 ± 0.22) versus placebo group (0.53 ± 0.25) was also similar in t test comparison ($p = 0.54$). Other covariate values are shown for HBD/DIC-positive patients stratified by treatment in **Table 3**. None of these were statistically significant. Taken together, these findings indicate the treatment groups are balanced on risk of death and other potential confounding variables.

To further confirm the results, the patients were divided into three groups including HBD/DIC positive ($n = 43$), HBD/DIC negative ($n = 470$), who have neither HBD nor DIC, and 217 who have either HBD or DIC positive but not both (HBD/DIC equivocal). There is a large significant difference in 28-day survival between these three groups, with HBD/DIC-positive patients having the lowest survival rate (54%), followed by HBD/DIC-equivocal patients (58%), and HBD/DIC-negative patients having the highest survival rate (77%; chi-square = 32.1; $p < 0.0001$; phi = 0.21).

In univariate analysis (using chi-square for categorical variables and t test for age), age ($p < 0.0001$), AKI ($p < 0.0001$), and ARDS ($p = 0.0066$) are associated with 28-day mortality, whereas gender ($p = 0.73$), infectious cause ($p = 0.66$), shock ($p = 0.22$), and sepsis cause ($p = 0.21$) are not. Therefore, age, AKI, and ARDS were used as covariates in examining the association of drug with 28-day mortality in HBD/DIC patients.

In the logistic regression model in HBD/DIC-positive patients, the drug was associated with a reduced rate of death in HBD/DIC patients, after controlling for covariates, with an adjusted odds of 28-day mortality 87% lower than HBD/DIC patients on placebo (odds ratio for death, 0.13 [0.03–0.71]; $p = 0.018$).

Also in the logistic regression model using all patients, the diagnosis × treatment interaction was significant ($p = 0.025$), indicating different drug effects on mortality, depending on HBD/DIC status. Treatment with anakinra was associated with increased incidence of 28-day mortality in HBD/DIC-equivocal patients when compared with that in controls, but anakinra therapy was associated with reduced incidence of 28-mortality in HBD/DIC-positive patients when compared with that in non-HBD/DIC controls. The

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TABLE 2. Characteristics and Associations of Patients Dichotomized Based on Presence or Absence of Hepatobiliary Dysfunction/Disseminated Intravascular Coagulation

	All ($n = 763$)	HBD/DIC ($n = 43$)	Non-HBD/DIC ($n = 720$)	p
Mean age (yr) (range)	57.9 (17–95)	52.3 (18–75)	58.3 (17–95)	**0.025**
Female sex (%)	313 (41)	20 (46.5)	293 (40.7)	0.523
Anakinra (recombinant interleukin-1 receptor antagonist) treatment (%)	510 (66.8)	26 (60.4)	484 (67.2)	NA
Mean survival day	21.4	16.5	21.7	**0.0004**
Predicated death (range)	0.346 (0.015–0.986)	0.553 (0.143–0.986)	0.345 (0.015–0.95)	**0.0001**
Alive in day 28	527 (69.1)	23 (53.5)	504 (70.0)	**0.0273**
Concurrent dysfunctions (%)				
Acute respiratory distress syndrome	194 (25.4)	9 (20.9)	185 (25.7)	0.590
Acute kidney injury	232 (30.4)	26 (60.5)	206 (28.6)	**0.0001**
Shock	613 (80.3)	41 (95.3)	572 (79.4)	**0.0089**

HBD = hepatobiliary dysfunction, DIC = disseminated intravascular coagulation.
Statistically significant $p = 0.05$. Boldface font indicates statistically significant values.

multicolinearity test found all variance inflation factor less than 1.2 for all predictors, indicating no issue with multicollinearity.

In the Cox regression analysis of HBD/DIC-positive patients only, treatment was significantly associated with time to death ($p = 0.0071$) with a hazard ratio of 0.28 (0.11–0.71) for anakinra versus placebo groups, after adjusting for age, AKI, ARDS, and death risk score. In Cox regression in the full sample, using six groups of HBD/DIC × treatment (HBD/DIC positive, HBD/DIC negative, or HBD/DIC equivocal by treatment [anakinra vs placebo]) with HBD/DIC-positive placebo treated as the reference group, the hazard ratio for the HBD/DIC-positive treatment group was 0.28 (0.12–0.68; $p = 0.0051$) after adjusting for age, AKI, ARDS, and risk score. This indicates that the HBD/DIC-positive patients who received the drug had a significantly reduced likelihood of 28-day mortality when compared with HBD/DIC patients in the placebo group. Kaplan-Meier survival estimates for the six combinations of HBD/DIC and anakinra demonstrate the survival advantage for the HBD-positive/anakinra group over the HBD-positive/placebo group begins on day 1 and becomes more apparent on day 5.

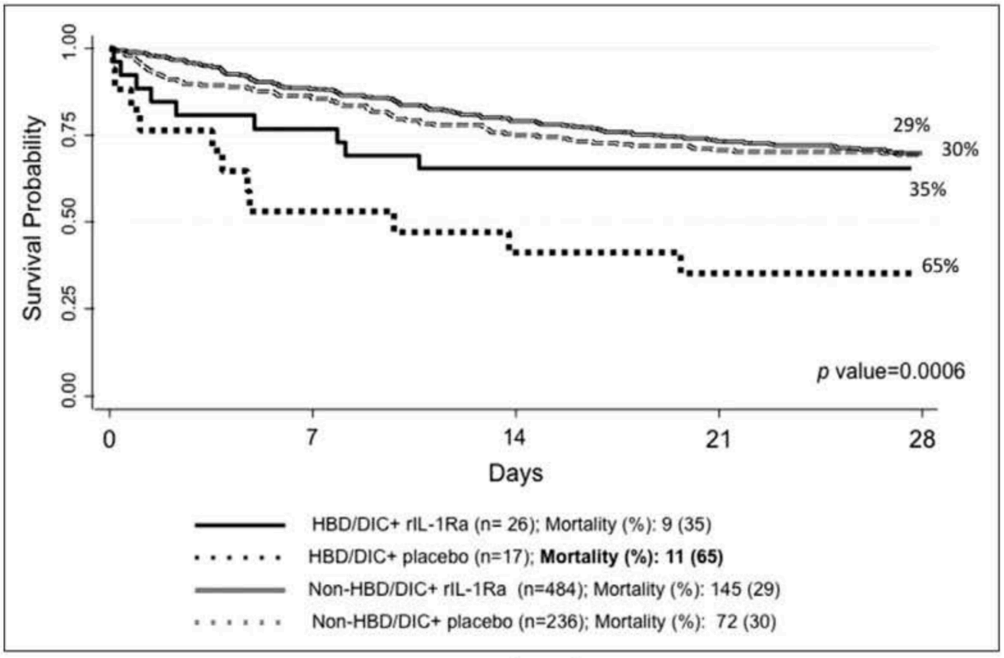

Figure 1. Treatment with interleukin-1 receptor antagonist (IL-1Ra) significantly improves 28-d survival in hepatobiliary dysfunction (HBD)/disseminated intravascular coagulation (DIC) sepsis, but does not change the outcome in non–HBD/DIC sepsis. rIL-1Ra = anakinra (recombinant interleukin-1 receptor antagonist), HBD/DIC group = patients with severe sepsis who demonstrate both HBD and DIC features, non–HBD/DIC group = patients with either HBD alone (no DIC), DIC alone (no HBD, or neither). Statistically significant $p = 0.05$.

DISCUSSION

The benefits of IL-1R blockade in modifying the detrimental effects of the inflammatory process have been demonstrated since 1984 (3); yet, the appropriate target population among sepsis/MODS patients who would benefit from treatment with anakinra has remained elusive. The recent recognition of and

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TABLE 3. Values of Pretreatment Variables in Treatment Versus Control Groups in Patients With Hepatobiliary Dysfunction/Disseminated Intravascular Coagulation

Variable	Treatment ($n = 26$)	Control ($n = 17$)	p
Age (yr)	49.6 ± 12.7	56.3 ± 19.4	0.18
Female sex (%)	12 (46.2)	8 (47.1)	0.95
Acute kidney injury (%)	17 (65.4)	9 (52.9)	0.41
Acute respiratory distress syndrome (%)	6 (23.1)	3 (17.7)	0.28
Risk of death	0.57 ± 0.22	0.53 ± 0.25	0.54

development of treatments for MAS may be informative because MAS and severe sepsis share many clinical, laboratory, and pathologic features, including elevated serum ferritin (28, 29), cytopenias, hepatic dysfunction, coagulopathy (28, 29), CNS dysfunction (30), tissue hemophagocytosis (31), and elevated macrophage expression of CD163+ cells throughout the reticuloendothelial system (27, 32). These shared clinical/laboratory features of MAS and severe sepsis occur in the context of a cytokine storm in which IL-1 plays a major role (2, 3, 6, 15).

Anakinra is a recombinant human IL-1Ra with a short half-life of about 3–4 hours (7), wide therapeutic range (7), and high safety profile (7). The use of anakinra as a treatment option for MAS was initially reported in 2006 (33) with subsequent case reports/series reporting the benefits of IL-1R blockade, mainly in children with MAS complicating systemic juvenile idiopathic arthritis and adults with Still disease (34–44). It is, therefore, plausible that a subset of severe sepsis patients develop MAS, and that the associated severe organ dysfunction is responsive to IL-1 blockade with anakinra.

In recent studies to develop validated case definitions for MAS, attributes of HBD (liver enzyme elevation) and DIC (hypofibrinogenemia and thrombocytopenia) are consistently among the most prevalent clinical features encountered in patients with presumed MAS (18, 20). Both HBD and DIC were readily assessed in the original anakinra/sepsis phase III study data, permitting the use of the presence of HBD and DIC as a marker for possible MAS in the current analysis.

Mortality rates of adult MAS have been reported as up to 60% in various studies (21, 22) comparable with the observed mortality rate noted in the HBD/DIC patients randomized to placebo. Treatment with anakinra reduced mortality in HBD/DIC patients when compared with the mortality rate of non-HBD/DIC patients, but did not lead to a meaningful difference in survival in non-HBD/DIC patients when compared with those randomized to placebo. These findings suggest severe sepsis patients with HBD/DIC may represent a state of cytokine storm similar to MAS and high mortality risk, who may be the most appropriate patients to receive immune modulating and cytokine-targeted therapies. The identification of the appropriate subgroup of sepsis patients to target for potentially life-saving anticytokine targeted therapy provides a rationale for future prospective investigations to verify the use of IL-1 blockade in sepsis associated with features of MAS.

When compared with activated protein C (45) as an intervention for sepsis, anakinra has no significant reported safety issues since introduction in the market in 2001 (46), with higher survival benefits in the selected population in this study and a lower number needed to treat. Although the subset analysis undertaken herein offers suboptimal power, these favorable findings merit future randomized studies to establish the benefits of anakinra in sepsis patients with features of MAS and MAS patients who have complicating sepsis.

CONCLUSIONS

The present findings support the possibility that anakinra treatment provides a survival benefit in septic patients with features of MAS. The safety and wide therapeutic margin of anakinra and the central role of IL-1 in the cytokine storm in severe sepsis patients merit reconsideration of this therapeutic as a potential treatment option in carefully selected groups of sepsis patients with attributes of MAS.

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MAJOR ARTICLE

Ebola Hemorrhagic Fever: Novel Biomarker Correlates of Clinical Outcome

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Background. Ebola hemorrhagic fever (EHF) outbreaks occur sporadically in Africa and result in high rates of death. The 2000–2001 outbreak of Sudan virus–associated EHF in the Gulu district of Uganda led to 425 cases, of which 216 were laboratory confirmed, making it the largest EHF outbreak on record. Serum specimens from this outbreak had been preserved in liquid nitrogen from the time of collection and were available for analysis.

Methods. Available samples were tested using a series of multiplex assays to measure the concentrations of 55 biomarkers. The data were analyzed to identify statistically significant associations between the tested biomarkers and hemorrhagic manifestations, viremia, and/or death.

Results. Death, hemorrhage, and viremia were independently associated with elevated levels of several chemokines and cytokines. Death and hemorrhage were associated with elevated thrombomodulin and ferritin levels. Hemorrhage was also associated with elevated levels of soluble intracellular adhesion molecule. Viremia was independently associated with elevated levels of tissue factor and tissue plasminogen activator. Finally, samples from nonfatal cases had higher levels of sCD40L.

Conclusions. These novel associations provide a better understanding of EHF pathophysiology and a starting point for researching new potential targets for therapeutic interventions.

Keywords. Ebola virus; biomarkers; hemorrhage; Gulu; hemorrhagic fever virus.

Ebola hemorrhagic fever (EHF) was first identified in 1976 during an outbreak in the Democratic Republic of the Congo (formerly known as Zaire). Since then, 21 additional outbreaks among humans have been recorded in sub-Saharan Africa. The largest of these outbreaks took place in the Gulu district of Uganda in 2000–2001 and was caused by Sudan virus (SUDV). This outbreak resulted in 425 cases, 216 of which were laboratory confirmed, and a 53% overall case-fatality rate [1].

The genus *Ebolavirus* includes 5 different viruses: SUDV, Tai Forest virus (TAFV), Reston virus (RESTV), Ebola virus (EBOV), and Bundibugyo virus (BDBV). EHF caused by EBOV has the highest fatality (57%–90%), followed by SUDV (41%–65%) and BDBV (40%); to date, RESTV infection has been asymptomatic in humans, and TAFV has only been identified in 2 human infections, both of which were nonfatal [2–4]. Clinical EHF is characterized by abrupt onset of fever, fatigue, headache, myalgia, and gastrointestinal distress 3–13 days after exposure to virus [5]. Many patients develop hemorrhagic manifestations, which has led to the term "hemorrhagic fever."

The samples collected during the Gulu outbreak have already yielded extensive information about EHF pathophysiology, including the observation that aspartate aminotransferase (AST), D-dimer, blood urea nitrogen (BUN), and creatinine levels are higher than normal, while calcium and albumin levels are lower than normal in samples from fatal EHF cases [6]. Elevated levels of the cytokines interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 10 (IL-10), and macrophage inflammatory protein 1β (MIP-1β) were also associated with fatal outcomes [7]. Additionally, human leukocyte antigen B67 (HLA-B67), HLA-B15, and marked CD8

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lymphopenia were associated with fatal outcome, and HLA-B7 and HLA-B14 were associated with nonfatal outcome [8, 9].

The goal of our study was to better understand the pathophysiology of EHF by correlating hemorrhagic manifestations and death with specific biomarkers. We measured levels of markers of immune function, endothelial activation, and coagulation in available patient serum samples, to determine whether alterations in levels of these biomarkers were associated with hemorrhagic manifestations, viremia, or death.

METHODS

Study Design

During the 2000–2001 Gulu outbreak, an international response team, including Centers for Disease Control and Prevention (CDC) staff, responded with clinical and technical assistance. Serum samples were frozen in liquid nitrogen in the field and have been stored that way since the time of the outbreak. For our analyses, samples were chosen to represent sex ratios, hemorrhagic manifestations, and death rates consistent with those observed during the outbreak (Table 1). Specimens were prioritized for novel analyses; serum chemistry analyses were performed on samples of sufficient volume. All samples were inactivated by γ irradiation before use.

Institutional Review Board (IRB)

IRB approval was obtained before initiating the study, and an exemption was granted by the CDC Human Research Protection Office.

Luminex-Based Assays

The following assays were purchased from Affymetrix (Santa Clara, CA) and performed according to the manufacturer's instructions: a 26-plex assay for granulocyte macrophage colony stimulating factor (GM-CSF), GROα, interferon α2 (IFN-α2), IFN-β, IFN-γ, IL-10, interleukin 12p70 (IL-12p70), IL-12p40, interleukin 1α (IL-1α), IL-1β, IL-1 receptor antagonist (IL-1RA), interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 5 (IL-5), IL-6, IL-8, IFN-γ–inducible protein 10 (IP-10), monocyte chemoattractant protein 1 (MCP-1), macrophage colony-stimulating factor (MCSF), MIP-1α, MIP-1β, soluble CD40 ligand (sCD40L), soluble E-selectin (sE-selectin), soluble Fas ligand (sFasL), tumor necrosis factor α (TNF-α), and vascular endothelial growth factor A (VEGF-A); a 2-plex assay for D-dimer and tissue plasminogen activator (TPA); a 5-plex assay for plasminogen activator inhibitor-1 (PAI-1), serum amyloid antigen (SAA), regulated on activation, normal T-cell expressed and secreted (RANTES), soluble intracellular adhesion molecule 1 (sICAM-1), and soluble vascular cell adhesion molecule (sVCAM-1); and single-plex assays for C reactive protein (CRP) and fibrinogen. Assays for ferritin and cortisol were performed as single-plex assays according to manufacturer's instructions (Millipore, Billerica, MA). 2-plex assays were performed for tissue factor (TF) and thrombomodulin according to the manufacturer's instructions (Millipore). For samples with values outside the upper end of the standard curve, additional dilutions were made as necessary to obtain accurate values for all analytes.

Table 1. Patient Characteristics

Characteristic	This Study (n = 86)	Gulu Outbreak (n = 425)
Age, y, range	6–60	0.25–81
Sex		
Female	56 (65)	267 (63)
Male	30 (35)	158 (37)
Any hemorrhagic manifestation	31 (36)	128 (30)
Death	41 (48)	224 (53)

Data are no. (%) of patients, unless otherwise indicated. Gulu outbreak data are from Okware et al [1].

Enzyme-Linked Immunosorbent Assays (ELISAs)

Mannose-binding lectin (MBL; Hycult Biotech, Plymouth Meeting, PA) and total immunoglobulin G (IgG; eBioscience, San Diego, CA) ELISAs were performed according to the manufacturers' instructions. For samples with values outside the upper end of the standard curve, additional dilutions were made as necessary to obtain accurate values for all analytes.

Serum Chemistry Analyses

A Piccolo comprehensive metabolic reagent disc was run on the Piccolo xpress Chemistry Analyzer (Abaxis, Union City, CA) to determine serum chemistry values for alanine aminotransferase (ALT), albumin, AST, alkaline phosphatase, calcium, chloride, creatinine, glucose, potassium, sodium, total bilirubin, total CO_2, total protein, and BUN. Only 132 of the 187 samples had sufficient volume for serum chemistry analyses.

Human Immunodeficiency Virus (HIV) Testing

The Multispot HIV-1/HIV-2 Rapid Test (BioRad, Hercules, CA) was used to assess the HIV status of the patient cohort. Two patients (ages 7 and 19 years) did not have sufficient sample for analysis.

Viremia Analysis via Measurement of RNA Copy Number

Total RNA was isolated from serum using the MagMAX-96 Viral RNA Isolation Kit on the MagMAX Express-96 Magnetic Particle Processor (Ambion, Grand Island, NY). In parallel, serial dilutions of a normal human serum specimen inoculated with a known titer of SUDV was subjected to RNA isolation and used to generate a standard curve. Real-time polymerase chain reaction was then performed as previously described [10].

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Statistical Analysis

Data on sex, age, date of symptom onset, viremia levels, hemorrhagic manifestations, HIV status, patient survivability, and date of sample collection were available for the 86 patients included in our study. Only samples taken 0–15 days from symptom onset were analyzed, to represent the normal course of the illness.

Analysis of variance (ANOVA) was conducted for each of the 55 analytes, using sex, age, day from symptom onset, viremia, death, hemorrhaging, and HIV status as the independent variables. All variables were converted to categorical variables (meaning that there was limited number of outcomes [ie, viremic vs not viremic]) because of the number of samples and the distribution of the numerical data, with the date from symptom onset converted to 3 categories (0–5 days, 6–10 days, and 11–15 days). An initial overall α of 0.25 was set, and the Bonferroni inequality was used to arrive at a critical P value of .0045 for the individual analytes. Using these criteria, 29 of the 55 analytes had statistically significant differences. Knowing the impact of multiple testing on the power of tests, the technique of Yoav and Hochbergs was used to control the false discovery rate to balance the power and type 1 error of the analyses [11]; this correction resulted in 10 additional analytes meeting the criteria for statistical significance. The independent variables were analyzed to measure colinearity, but no significant variance inflation was observed.

Ultimately, our study included 161 samples, each described by 9 categorical variables. Each sample was analyzed for 41 different analytes (one sample was insufficient to perform a subsequent analysis of 2 analytes). An analysis of metabolic information was attempted for all samples, but only 132 samples had sufficient volume for analyzing 14 additional analytes.

Eight analytes (IL-12p70, IL-12p40, IL-2, IL-4, IL-5, sFasL, IFN-γ, and IFN-α2) had statistically significant correlations but were excluded from analysis because $\geq$30% of the samples had a value below the limit of detection. We therefore did not attempt to make conclusions based on data that could have been affected by degradation over time.

Model Selection

After the initial ANOVA, 27 significant analytes were selected for further analysis. Stepwise regression for each significant analyte was used to find the best model, excluding insignificant variables at an α of 0.05. Particular attention was paid to those analytes in which age, death, viremia, hemorrhaging, and HIV status were significant. Ten analytes were significant for age, 16 for death, 7 for viremia, 8 for hemorrhaging, and 2 for HIV status. We compiled the analytes that were significantly different for these variables and calculated the means and standard error for each interval.

Finally, 2 additional statistical tests were performed to measure the relationships between death and both viremia levels and the presence of hemorrhaging. The distribution of the viremia levels was clearly nonnormal, so the Mann–Whitney U test was used to determine differences in viremia for patients with fatal and those with nonfatal outcomes. Patients with no measurable viremia were excluded from analysis. The survivability of patients with and those without hemorrhaging was also examined, but no significant differences were found.

RESULTS

Study Population

The study included 86 EHF patients from the 2000–2001 Gulu outbreak that were representative of the outbreak as a whole. As noted in previous outbreaks, more women were affected than men, likely because women are commonly caretakers, a known risk factor for contracting EHF. Thirty-six percent of the patients in this study had hemorrhagic manifestations (defined as any of the following: vomiting blood, blood in the stool, or bleeding from the gums, skin, or eyes), and approximately half of the patients died, consistent with reported death rates for SUDV (Table 1). One limitation to our study was that the limited sample volume influenced our selection of samples because we only selected patients for whom there were sufficient sample volume to complete the desired testing. This may have biased our data slightly toward the nonfatal group.

In the filovirus field, it has generally been assumed that immune system deficiencies associated with HIV infection would likely negatively impact EHF outcome. As the HIV status of EHF patients has never been determined previously, HIV testing was conducted on patients included in this study. None of the patients <18 years of age were HIV positive. Of the 67 adults (ages 18–60 years), 12 (17.9%) were HIV positive. This rate is roughly consistent with data on HIV prevalence in the Gulu region of Uganda during the time of the outbreak; 12% of women attending antenatal clinics were reported to be HIV positive [12]. Of the 12 HIV-positive adults, 5 were male and 7 were female. Although $CD4^+$ T-cell counts were unknown in these patients, 50% of the HIV-positive adults (3 males and 3 females) had fatal outcomes. This mortality rate was approximately the same as that in the total adult population, suggesting that an individual's HIV status did not significantly affect outcome during SUDV infection.

Study Design

Fifty-five different biomarkers were assessed in 187 serum samples in this study. Thirty-six patients had $\geq$2 serial samples collected 1–15 days after onset of symptoms; composite data rather than individual patient data are presented, to provide the most objective data for analysis. Supplementary Table 1 provides the statistical significance for each biomarker tested and its relationship to the examined variables. The data presented in this article represent the statistically significant associations observed

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Table 2. Coefficient of Variance (CV) Between Repeat Chemistry Measurements After 12 Years of Storage in Liquid Nitrogen

CV	ALT	AST	BUN	CRE	TP	ALB
Percentage difference, mean (range)	2.3 (0–7.4)	1.5 (0–4.4)	1.7 (0–7.1)	2.2 (0–5.4)	0.96 (0–2.9)	0.75 (0–2.8)

Abbreviations: ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CRE, creatinine; TP, total protein.

between a given biomarker and death, hemorrhagic manifestations, or viremia.

Serum Chemistry Analysis and Viremia

Serum chemistry analyses were performed on many patients as part of their clinical management during the 2000–2001 outbreak. These data were still available, allowing for a direct assessment of the stability of several metabolic analytes after 12 years of storage in liquid nitrogen. Many analytes were strikingly stable, as shown by a maximal coefficient of variance of 7.4% between the original measurement and the repeat measurement for ALT, AST, BUN, creatinine, total protein, and albumin levels (Table 2). The more labile components, CO_2 and electrolytes, were excluded from our analysis.

Previously reported associations between fatal outcomes and AST, BUN, creatinine, and albumin levels [6] were again seen in our study (data not shown) [6]. Higher levels of viremia were associated with a fatal outcome, as previously demonstrated [10] (Figure 1).

Cytokines and Chemokines

Cytokines and chemokines are a diverse, extensively studied group of proteins that modulate the immune response. We analyzed 25 different cytokines and chemokines. IL-1α, IL-1RA, IL-6, MCP-1, MCSF, and MIP-1α levels were all elevated in patients with a fatal outcome (Figure 2*A*–*E* and 2*G*).

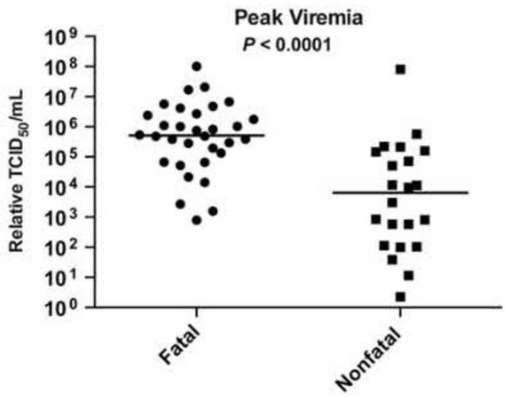

Figure 1. Viremia as a function of clinical outcome. Patients with fatal outcomes had higher viral loads. $TCID_{50}$, median tissue-culture infective dose.

Previously published data have shown associations between MIP-1β, IL-6, IL-8, and IL-10 levels and fatal outcomes in SUDV-infected patients [7]. All of these prior associations were observed in our study. However, IL-8 levels were only elevated very late in the infection in patients with a fatal outcome (data not shown); MIP-1β levels tended to be higher in patients with fatal outcomes but did not meet the cutoff for statistical significance; and IL-10 levels demonstrated an age-specific association with death. GRO-α levels, similar to IL-8 levels, were only elevated late during infection in patients who died (data not shown). IL-1α, MCSF, and IP-10 levels were elevated in samples with documented viremia, compared with samples with no detectable virus, but this finding was independent of their relationship with death (data not shown). MCSF, MIP-1α, and IP-10 levels were also elevated in patients who developed hemorrhagic manifestations (Figure 2*F*, 2*H*, and 2*I*). Unexpectedly, elevated levels of sCD40L correlated with nonfatal outcomes, a first for any report of EHF (Figure 2*J*).

Biomarkers of Inflammation

The acute phase response refers to a constellation of host responses that occur following infection and in other inflammatory diseases [13]. These responses are classically triggered by proinflammatory cytokines, such as IL-6, IL-1, IFN-γ, and TNF-α. Increased liver production of CRP and SAA and decreased production of albumin are common consequences of the acute-phase reaction. In addition, hypergammaglobulinemia and hyperferritinemia are often seen. These markers of inflammation are used clinically in many disease processes to help in diagnosis and to follow a patient's response to therapy. CRP levels were within the normal range in all samples and were excluded from further analysis. Ferritin levels were elevated in patients with fatal outcomes and in those with hemorrhagic manifestations (Figure 3*A* and 3*B*). Ferritin levels were also associated with viremia, independent of the associations with death and hemorrhage (data not shown). Ferritin levels were higher in HIV-positive patients than in HIV-negative patients (data not shown); this has been previously noted specifically in association with advanced HIV disease [14, 15]. HIV-positive patients also had higher total IgG levels than HIV-negative patients (data not shown), a well-documented phenomenon [16]. Finally, levels of sICAM, a marker of endothelial activation [17], were elevated in patients with hemorrhagic manifestations (Figure 3*C*)

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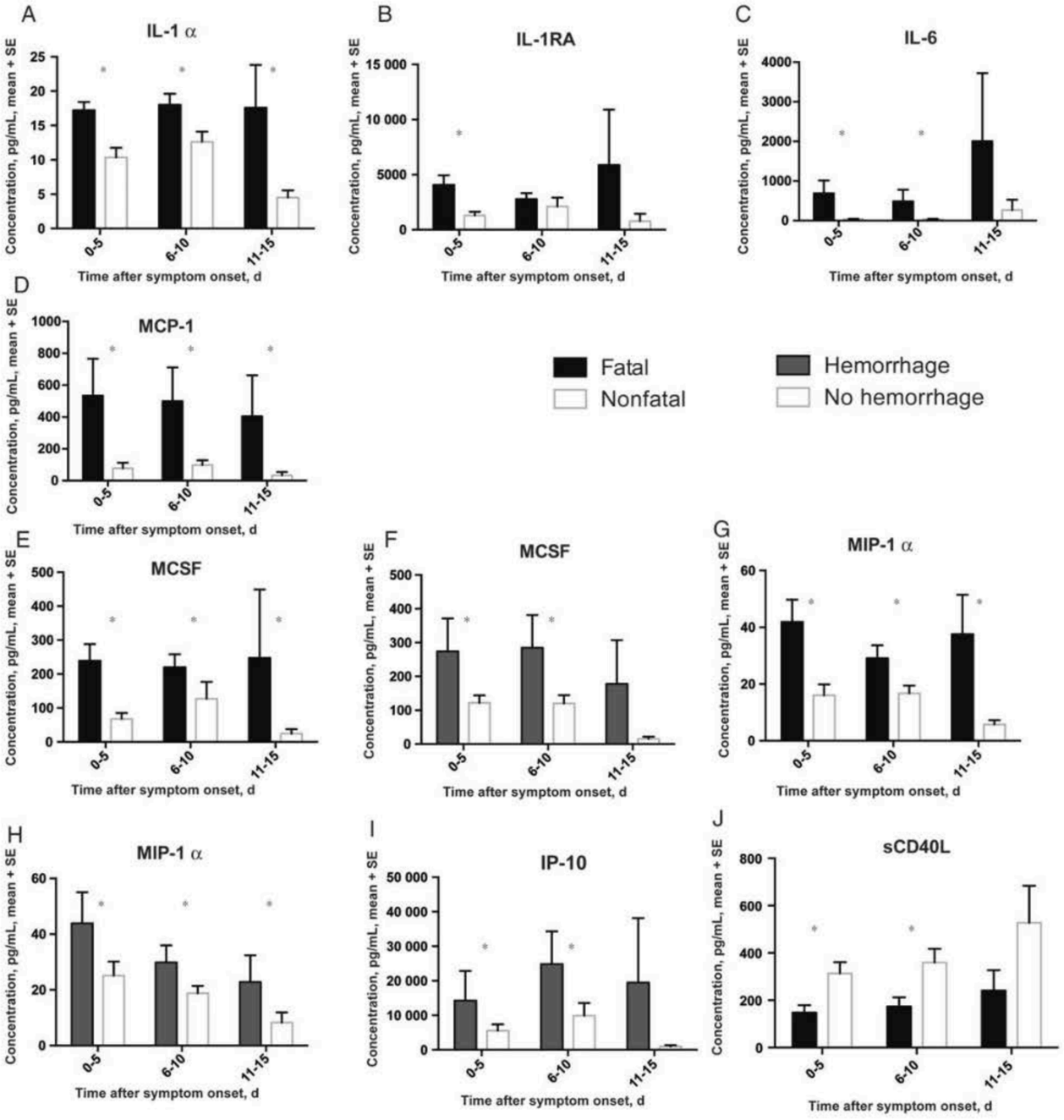

Figure 2. Cytokines and chemokines associated with a fatal outcome (black bars) or hemorrhagic manifestations (red bars). *$P \leq .05$. Abbreviations: IL-1α, interleukin 1α; IL-1RA, interleukin 1 receptor antagonist; IL-6, interleukin 6; IP-10, interferon γ–inducible protein 10; MCP-1, monocyte chemoattractant protein 1; MCSF, macrophage colony-stimulating factor; sCD40L, soluble CD40 ligand; SE, standard error.

Biomarkers of Coagulopathy

The frequency of hemorrhagic manifestations during EHF warranted an examination of the measureable factors that control hemostasis. The delicate balance of coagulation and fibrinolysis is depicted in a simplified model in Figure 4. In this study, PAI-1, fibrinogen, TPA, D-dimer, thrombomodulin, and TF levels were measured in all patient samples. TPA, D-dimer, thrombomodulin, and TF levels were all statistically significant in our analysis. Thrombomodulin levels were elevated in patients with fatal outcomes and in patients with hemorrhagic manifestations (Figure 4*A* and 4*B*). D-dimer levels were elevated in patients with a fatal outcome (Figure 4*C*), as previously reported [6]. TPA levels were higher in viremic patients (Figure 4*D*). TF levels were within the normal range in all samples

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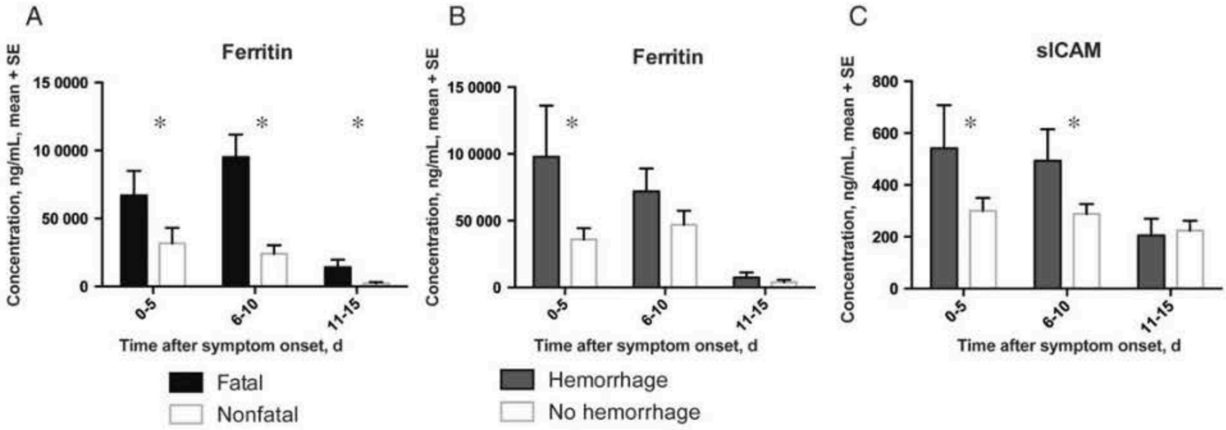

Figure 3. Markers of inflammation associated with a fatal outcome (black bars) or hemorrhagic manifestations (red bars). $^{*}P \leq .05$. Abbreviations: SE, standard error; sICAM, soluble intracellular adhesion molecule 1.

tested but were slightly higher in samples with detectable viremia than in those without detectable viremia (Figure 4*E*). Notably, TF levels were not associated with hemorrhagic manifestations or death.

DISCUSSION

We found associations between multiple proinflammatory cytokines and increased death rates, as previously shown in humans [7, 18–20] and EBOV-infected nonhuman primates [21], consistent with the idea of systemic inflammation contributing to a fatal outcome. We also found several novel associations, most strikingly, the association between increased sCD40L levels and nonfatal outcomes. Since sCD40L is largely produced by activated platelets [22], increased sCD40L levels could represent ongoing repair of altered endothelium by activated platelets. Additionally, nitric oxide (NO) inhibits platelet activation [23], and elevated NO levels have been reported in fatal EHF cases [9]. Therefore, the deleterious effects of NO could be related to inhibiting platelet activation, in addition to its vasodilatory effects. Effective repair of altered endothelium by activated platelets could lead to improved hemodynamic stability in patients and, thus, improved clinical outcomes.

Ferritin, another biomarker we examined, is a well-established acute-phase reactant that has been associated with severe disease in both dengue and Crimean Congo hemorrhagic fevers [24, 25]. Ferritin is the intracellular storage form of iron, and its levels increase during times of stress or inflammation. Ferritin levels were higher in samples from patients with EHF who had fatal outcomes, detectable viremia, and hemorrhagic manifestations; thus, ferritin could prove to be a useful clinical maker of EHF severity.

The striking clinical manifestation of hemorrhage during historic EHF outbreaks led to the naming of the disease as Ebola *hemorrhagic* fever, which has resulted in the perception that hemorrhage occurs in most patients and is associated with fatal outcomes. However, published data from the 3 largest EHF outbreaks, caused by EBOV (Kikwit, Democratic Republic of the Congo, 1995), SUDV (Gulu, Uganda, 2000), and BDBV (Bundibugyo, Uganda, 2007), demonstrate hemorrhagic manifestations in only 41%, 30%, and 46.5% of total cases, respectively [1, 3, 26]. Furthermore, no significant correlation between hemorrhage and death has been found in these same outbreaks of EBOV and BDBV infections. Consistently, we found no statistically significant differences in survival between patients with and those without hemorrhage ($P = .23$). Therefore, it is not surprising that, of the 6 different coagulation and fibrinolysis markers we assessed, only thrombomodulin was associated with both fatal outcome and hemorrhagic manifestations. Thrombomodulin is a marker of endothelial activation/dysfunction and, as discussed below, has many functions besides inhibiting coagulation. There is a common perception, not only in EHF but also in routine sepsis, that correction of coagulopathy will improve survival outcomes. However, therapeutic interventions that have focused on targeting coagulopathy in nonhuman primate models of EBOV only moderately increased survival [27, 28], and use of activated protein C in septic patients in the intensive care unit has been similarly disappointing [29]. A large focus has been placed on understanding the role of TF in EHF, given the marked increases in TF expression seen in monocytes in nonhuman primates infected with EBOV [30]. However, when TF levels were examined in patients from this outbreak, which was caused by SUDV, none of the patients had elevated levels of TF outside of the normal range, and there were no associations between TF levels and death or hemorrhage. These data are limited by our ability to only sample plasma TF versus monocyte-associated TF, although plasma and monocyte TF levels correlated in a human study of

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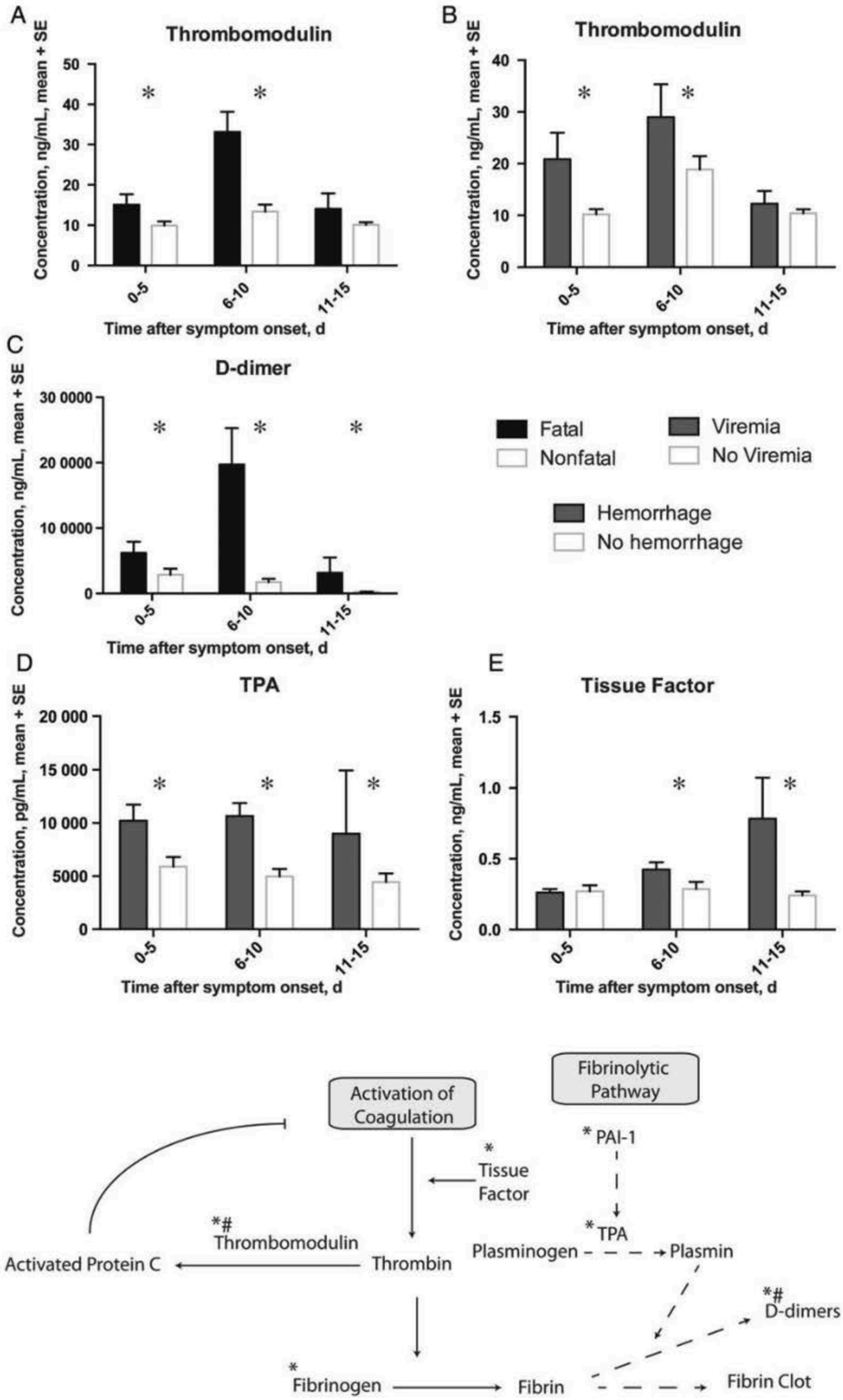

Figure 4. Markers of coagulation and fibrinolytic pathways associated with a fatal outcome (black bars), hemorrhagic manifestations (red bars) or viremia (blue bars). *$P \leq .05$. A simplified model of the coagulation/fibrinolytic pathway is also depicted. Proteins that are known to play a major role in the pathways are noted. Arrows indicate activation while bars indicate inhibition. *, analytes assessed in the study; #, analytes associated with death.

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thrombosis [31]. Finally, the nonhuman primate studies were performed with EBOV, whereas the patients in this study were infected with SUDV, so these TF differences could be virus or host related.

Thrombomodulin is released by an activated endothelium and functions as an anticoagulant by activating protein C when present in the microenvironment on the surface of endothelial cells. In our study, thrombomodulin levels were elevated in samples from patients who died and from those with hemorrhagic manifestations, the latter being consistent with its anticoagulant function. Thrombomodulin has several protein domains that are responsible for its anticoagulant activity, but it also contains a C-type lectin domain that has antiinflammatory activities, including maintaining endothelial integrity [32]. The fact that EBOV GP protein binds to other C-type lectins [33–35] makes the thrombomodulin C-lectin domain an attractive subject for further study. If thrombomodulin's C-lectin domain–mediated functions are inhibited by association with viral GP, this could provide a possible explanation for how these viruses are able to disrupt endothelial function without causing direct cytopathic effects. Alternatively, overactivation of endothelial cells, leading to shedding of thrombomodulin into the plasma, could result in a loss of thrombomodulin's stabilizing function at endothelial junctions, contributing to vascular leakage.

MCSF, MIP-1α, IP-10, sICAM, and thrombomodulin levels were all associated with hemorrhage in our study, and on the basis of findings in the literature, their activities can all be correlated with leukocyte/endothelium interactions. In one possible model, these cytokines could recruit leukocytes to areas of inflammation, and production of adhesion molecules such as ICAM would facilitate leukocyte adhesion, rolling, and diapedesis. Activated endothelial cells would then release thrombomodulin into the bloodstream, so it would no longer be present in the microenvironment to activate protein C and coagulation, nor would it be present to stabilize the endothelium. This would leave an activated, leukocyte-enriched, procoagulant endothelium, causing dysregulated hemostasis, which could manifest clinically as hemorrhage.

Future pathogenesis models could evaluate the possible role of activated platelets in improving clinical outcomes by therapeutic administration of platelets, inhibitors of NO synthetase, or active sCD40L to infected animals. Finally, the potential roles of thrombomodulin C-lectin domains in modulating EHF outcomes should be further studied at the molecular level to determine whether viral glycoproteins interact with thrombomodulin. If this proves to be the case, evaluating the role of thrombomodulin C-lectin domains in animal models would be warranted.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflict of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Editorial

Does Viral Hemorrhagic Fever Represent Reactive Hemophagocytic Syndrome?

The recent Ebola virus disease (EVD) outbreak is the largest in our planet's history. The epidemic is centered in West Africa, but isolated cases are being recognized across continents and represent a global health crisis[1]. The mortality rate, once infected in non-hospitalized patients, is near 70%[2]. Over 11,000 individuals have died in the recent outbreak, and this likely represents an underreported figure. In addition to the ever-rising death toll, children in West Africa are being orphaned at an alarming rate. With no proven vaccine or curative therapy currently available and the possibility for spread to tens of thousands of individuals or more, there is a desperate and urgent need for novel therapy to combat this deadly disease, yet clinical trials are just getting under way.

Clinical Features of Viral Hemorrhagic Fever (VHF)

Ebola virus infection, following an incubation period of 2 to 21 days, is clinically characterized initially by fever, myalgia, headache, weakness, and abdominal pain. This is frequently followed by vomiting, diarrhea, and occasionally by bruising and bleeding (about 1 in 5 infected); thus the terminology, Ebola hemorrhagic fever.

The hemorrhagic complications seen in Ebola virus infection are also described in other specific virus families by the US Centers for Disease Control, including arenaviruses, filoviruses, bunyaviruses, and flaviviruses; hence, the term viral hemorrhagic fever is used to describe the severe multiorgan dysfunction syndrome (MODS) in a substantial number of infected individuals[3]. VHF shares striking similarities with hemophagocytic syndromes (HPS), both familial hemophagocytic lymphohistiocytosis (HLH) and reactive hemophagocytic syndrome (rHPS, also called macrophage activation syndrome, or MAS), which include the following: prolonged fever, coagulopathy, hyperferritinemia, liver enzyme elevations, increased serum interleukin 1 (IL-1) levels, thrombocytopenia, and central nervous system (CNS) dysfunction[4,5] (Table 1)[6]. Moreover, it has recently been elegantly shown in samples from the 2000–2001 Ebola outbreak of Sudan virus-associated VHF that hyperferritinemia, a hallmark of rHPS, was associated with hemorrhage and death[4].

Like Ebola virus, rHPS has been reported in association with other hemorrhagic fever viruses, such as Crimean-Congo and Dengue, with similar clinical (e.g., MODS including CNS dysfunction and coagulopathy)[5,7,8] and pathologic findings (e.g., hemophagocytosis)[9,10]. As in Ebola virus infection, hyperferritinemia has been observed to be a marker of hemorrhagic manifestation (and adverse outcome) in Dengue as well[11]. Collectively, features of VHF are seen in a substantial fraction of patients with certain viral illnesses such as Ebola virus, Dengue fever, Crimean-Congo fever, and other similar viruses. The clinical-pathological characteristics of the corresponding hemorrhagic fevers demonstrate striking resemblance with rHPS complicating viral illnesses. Despite the nonspecific nature of these findings, the constellation of these manifestations taken together is highly suggestive of rHPS. Thus, it can be speculated that VHF may in fact represent rHPS complicating the underlying viral illness.

Table 1. Similarities between hemophagocytic lymphohistiocytosis (HLH) 2004 criteria and features of viral hemorrhagic fever (VHF). Adapted from Chatham and Cron. The Rheumatologist 2014;8:1,21–23[6]; with permission.

HLH-04 Criteria	Features of VHF
Fever	Routinely
Splenomegaly	Sometimes
Bicytopenia or pancytopenia	Frequently
Hypertryglyceridemia or hypofibrinogenemia	Both have been reported for some hemorrhagic fever viruses
Hemophagocytosis on biopsy	Yes, for the viruses studied
Hyperferritinemia (> 500 ng/ml)	Yes, often > 10,000 ng/ml
Elevated soluble CD25	Yes, for the viruses studied
Absence of decreased natural killer cell function	Poorly studied to date

Pathophysiology of Hemophagocytic Syndromes

Our basis of understanding hemophagocytic syndromes, HLH and rHPS, is primarily derived from the study of familial HLH. Patients with familial HLH have homozygous or compound heterozygous defects in cytotoxic granule function through either defective granule contents, e.g. perforin, or defective granule transport and fusion machinery[12,13]. Work in genetic mouse models of familial HLH has led to a conceptual paradigm wherein defective cytotoxic killing by CD8 T cells and natural killer cells during infection leads to an inability to clear infection, and subsequent accumulation of infectious antigen[14,15]. This accumulation of antigen results in overstimulation of the frustrated lymphocytes, copious release of cytokines (a cytokine storm), and the resulting multiorgan pathology[13]. Such situations may arise when cytotoxic granule function is genetically intact as well[16]. Additionally, patients with systemic lupus erythematosus, systemic juvenile idiopathic arthritis, and

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other chronic inflammatory diseases may have more subtle abnormalities in cytotoxic immune cell function, perhaps exacerbated by haploinsufficient or hypomorphic genetic lesions in the perforin pathway[13]. These same patients are at risk for developing rHPS given the appropriate triggers as well.

Importantly, not all patients with rHPS demonstrate defects in cytotoxic killing, suggesting other mechanisms can lead to cytokine storm and HPS as well. For instance, in mice, excessive Toll-like receptor 9 stimulation can lead to a perforin-independent — and indeed lymphocyte-independent — cytokine-driven HPS[16]. Given the clinical overlap between many features of VHF and rHPS, it is intriguing to speculate whether similar pathophysiology is at play at least in part in VHF. This might be conceived as occurring as a result of excessive immune activation, as in the Toll-like receptor 9 model, or perhaps due to a subset of patients with insufficient cytotoxic cell activity, leading to viremia and resultant cytokine storm, suggested for some systemic juvenile idiopathic arthritis patients with MAS[12].

Implications for Treatment of VHF as rHPS

Traditional treatment protocols for familial HLH presenting during infancy differ from management options for rHPS, and include etoposide-based chemotherapy, followed by bone marrow transplantation. This has lowered the 5-year mortality rate from 100% to about 45%. By comparison, in general, therapy for children with rHPS includes a less toxic immunosuppressive/immunomodulatory approach, frequently with high-dose corticosteroids and cyclosporine. The therapeutic guidelines in adults with rHPS who have other comorbidities remain less well defined. More recently, the development of biologic therapeutics targeting proinflammatory cytokines has allowed for additional treatment options in treating rHPS. Novel approaches to treating rHPS include targeting of tumor necrosis factor, IL-1, and IL-6 pathways[12,17]. The most well-reported biologic agent treatment responses for rHPS focus on the use of anakinra, a recombinant human IL-1 receptor antagonist (IL-1Ra)[12].

It is well known in the sepsis literature that the host immune response rather than the pathogen is largely responsible for MODS[18]. Nevertheless, immunosuppressive treatment paradigms for familial HLH and rHPS struggle with the fact that infection is often a trigger of disease, as well as a comorbidity of treatment. Immune modulation is often required in the face of infection to prevent mortality from the primary immune dysfunction. In the case of Dengue virus-induced rHPS, there are multiple reports of corticosteroids and intravenous immunoglobulin (IVIG) used to successfully walk the fine line between infection and immune dysfunction[19,20]. Similarly, 12 patients with Crimean-Congo hemorrhagic fever were successfully treated with high-dose corticosteroids and IVIG[21]. Treating VHF as rHPS strikes at the epitome of this balance, particularly considering that Ebola virus causes a suppression of interferon-induced immunity through viral protein 24 binding to STAT1 and antagonizing interferon signaling[22].

Whether Ebola virus infection will respond to immunosuppression like other VHF viruses, however, is less clear. For example, massive lymphocyte apoptosis has been reported during Ebola infection, questioning the potential benefits of further suppressing the lymphocyte population[23]. There are also data reporting increased proinflammatory cytokine levels in asymptomatic compared to symptomatic Ebola-infected individuals[24], as well as fatal cases of Ebola having lower levels of IL-1 compared to survivors[25]. In this same report, antiinflammatory mediators, such as IL-1Ra and IL-10, were associated with poor prognosis[25]. Nevertheless, it is possible that the increased serum levels of IL-1RA and IL-10 reflect the immune system's attempt to dampen the overexuberant immune response in the sickest Ebola-infected patients experiencing MODS. Indeed, blockade of IL-10 was required to demonstrate hemophagocytosis in a murine model of rHPS[16]. Moreover, the concept of immune suppression of Ebola VHF has been recently considered by others[26].

In considering VHF as a viral infection complicated by rHPS, prudent choice of immune modulation would be of utmost importance. Borrowing from the rHPS experience, IL-1 blockade is emerging as an efficacious intervention that has not demonstrated any increased infectious risk or mortality in clinical trials of sepsis[27]. Although the beneficial role of anticytokine therapy targeting IL-1 in rHPS has yet to be formally validated in clinical trials, the efficacy and safety of this intervention in a disorder associated with up to 60% reported mortality has nonetheless become increasingly recognized[28]. There is now sufficient clinical and laboratory evidence that VHF may reflect viral-induced rHPS. It therefore follows that using a targeted, low infectious risk immunomodulatory approach, such as short term IL-1 blockade and/or IVIG and corticosteroids, might be an unrecognized opportunity to alter the outcome of this disease[26]. Indeed, use of immunomodulatory versus aggressive chemotherapeutic dosing was associated with increased survival in a large cohort of rHPS patients[29]. Secondary consideration of the potential use of other immunomodulatory approaches for rHPS, such as IL-6 blockade or other cytokine pathways that are emerging from other research models, may play a role in our armamentarium as well[17,30].

Given an expanding Ebola epidemic in which mortality nears 70%, a novel approach in management is worthy of investigation, particularly for those individuals who have VHF and features of rHPS. In this case, incorporating treatment of the overly exuberant host immune response to the pathogen as part of the supportive care strategy may provide an opportunity to substantially decrease mortality.

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Ebola virus disease has features of hemophagocytic lymphohistiocytosis syndrome

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The 2014 epidemic of Ebola virus disease (EVD) in several West African countries has mortality rates of up to 70%, as reported by WHO. The exponential increase in the number of infections and deaths, coupled with the absence of specific preventive and therapeutic strategies, represents one of the biggest global health challenges of this millenium. Insights in the pathophysiology and treatment of EVD are thus urgently needed. In this paper, we postulate that EVD has the characteristics of hemophagocytic lymphohistiocytosis (HLH) syndrome (syn., macrophage activation syndrome). The identification of HLH as part of severe EVD, however, brings the possibility for a pathophysiologically targeted approach of therapy; special promise is represented by cytokine-directed therapy in the form of recombinant interleukin-1 receptor antagonist (anakinra). Treatment with anakinra has the advantage to interrupt the deleterious IL-1-mediated hyperinflammatory loop in macrophage activation syndrome, and it has been shown to be associated with remarkable effectiveness and an excellent safety record.

CLINICAL SYNDROME

After an incubation period of 2–21 days, the symptoms of EVD present that resemble flu, including chills, fever, myalgia, and general malaise. As the disease progresses, abdominal complaints such as anorexia, nausea, vomiting, abdominal pain, diarrhea, as well lethargy, headache, and hypotension develop. Also bleeding may develop in 30–50% of patients, however, the severity varies. Bleeding is not *per se* associated with fatal outcome. In severe and fatal forms, death occurs between 6 and 16 days after symptoms appeared because of shock, seizures, severe metabolic complications, and bleeding. In lethal cases, a high blood viral load is seen, accompanied by a significant increment in blood neutrophils, drop in numbers of lymphocytes and platelets, and coagulation abnormalities (1).

Laboratory findings include initially leukopenia (which may drop to 1000 cells/μL) with lymphopenia, while neutrophilia develops thereafter with a left shift with atypical lymphocytes, thrombocytopenia (50000–100000 cells/μL), elevated liver functions with higher aspartate aminotransferase levels compared to alanine aminotransferase, hyperproteinemia, and proteinuria. Diffuse intravascular coagulopathy may be present because of prolonged prothrombin and partial thromboplastin times and the presence of fibrin split products. When a secondary bacterial infection eventually may develop, elevated numbers of white blood cells are documented. In fatal cases, a high blood viral load is seen, accompanied by a significant rise in blood neutrophil count, a progressive drop in lymphocyte and platelet counts, and diffuse coagulation abnormalities (1).

PATHOGENESIS OF EBOLA VIRUS DISEASES

Little is known about the pathology and pathogenesis of EVD in humans. Ebola virus has a broad cell tropism infecting not only immune cells like monocytes, macrophages, and dendritic cells but also various epithelial cells as well as endothelial cells, fibroblasts, hepatocytes, and adrenal cortical cells. Data from experimentally infected non-human primates using Zaire Ebola virus suggest that monocytes, macrophages, and dendritic cells are probably preferably infected at first, whereby these cells subsequently play a significant role to disseminate the virus by spreading it from the side of infection to draining lymph nodes and then to liver and spleen. The tropism of Ebola virus for lymphoid tissue, the liver, and the adrenal gland is important for the pathogenesis as adreno-cortical dysfunction by Ebola virus infection could contribute to the development of shock that characterizes the late stage of disease.

Studies in non-human primates and lethal human EVD show significant lymphocyte reduction and necrosis in spleen, thymus, and lymph nodes, although a significant cellular inflammatory reaction is lacking in these or other infected tissues. The apoptosis that is generally noticed in lymphocytes in infected humans and in non-human primates may clarify the increasing peripheral lymphopenia and tissue lymphoid depletion at post-mortem studies. T-lymphocyte and natural-killer cell populations are mostly reduced in macaques infected with Zaire Ebola virus (2) while human studies indicate that the number of NK cells generally decrease while fatal cases are characterized by especially reduced T-cells counts (3). In addition, blood D-dimer levels can be found in EVD, but are fourfold higher in non-survivors, indicating disseminated intravascular coagulation (DIC) as an important component of severe EVD (4).

HOST IMMUNE RESPONSE DURING EVD

A substantial release of pro-inflammatory mediators and vasoactive elements has

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been shown in patients with severe EVD, contributing to inflammation and coagulation (5). In addition, an ineffective host response causes a high viral load that contributes to a pro-inflammatory state. The release of pro-inflammatory cytokines by monocytes and macrophages is deleterious by causing endothelial leakage, hypotension, DIC, and multiorgan failure, hence a syndrome that closely resembles septic shock. A recent study using 86 samples from the 2000–2001 Uganda outbreak correlated inflammatory biomarkers with clinical outcome, and found that death was associated with elevated concentrations of cytokines (IL-1α, IL-1RA, IL-6, MCP-1, MCSF, MIP-1α) and elevated D-dimer levels, as was found by others. Interestingly, this study also found elevated thrombomodulin and ferritin concentrations, being associated with hemorrhage and death (6).

Host immune responses have also been studied in non-human primates, the current gold standard experimental model of EVD. Strongly up-regulated cytokines/chemokines, including IL-1α, IL-1Ra, IL-6, MCP-1, MIP-1α, IL-15, and IL-18 were reported in experimentally infected macaques (7). Another striking finding was that, although initially fibrinogen levels increase, in the terminal stages of the infection plasma fibrinogen levels were found to decrease rapidly. In humans, plasma fibrinogen levels are generally low, as diffuse intravascular coagulation is very common (1).

HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

Hemophagocytic lymphohistiocytosis (also called macrophage activation syndrome) is an often fatal syndrome of exaggerated but ineffective inflammatory response, characterized by excessive macrophage and T-cell activation and an impaired capacity of NK cells and cytotoxic T cells to kill target cells (8). There are eight diagnostic criteria including fever, splenomegaly, cytopenias affecting at least two of the three lineages in the peripheral blood, hyperferritinemia >5000 μg/L, hypertriglyceridemia or hypofibrinogenemia, hemophagocytosis in the bone marrow, spleen, or lymph nodes, low or absent NK cell activity determined by 51-Cr release assay, and high levels of sCD25 (soluble interleukin-2 receptor). Five of these eight diagnostic criteria are required for diagnosis. Both familial (genetic due to mutations in the perforin pathway) (9) and acquired (reactive) forms of the syndrome are recognized, the latter being triggered by infectious, autoimmune, or neoplastic conditions. Most cases of virus-induced HLH are due to viruses of the herpes family, although a number of other viruses, including parvovirus B19, measles, mumps, influenza, enterovirus, and dengue have also been reported in association with HLH. The key event in HLH is a "cytokine storm" resulting in pancytopenia and hypertriglyceridemia. In addition, the macrophage activation leads to sustained and uncontrolled macrophage and histiocyte infiltration, resulting in hepatosplenomegaly, release of plasminogen activator promoting hypofibrinogenemia, up-regulation of hemoxygenase, and increase in ferritin levels.

HYPOTHESIS: SEVERE EVD IS CHARACTERIZED BY OVERWHELMING MACROPHAGE ACTIVATION SYNDROME

Based on the clinical and pathophysiological characteristics of EVD, we hypothesize that infection with Ebola virus is associated with overwhelming macrophage activation in the setting of a secondary HLH. Strong arguments for this hypothesis are the presence of fever, cytopenia, hypofibrinogenemia, low NK cells, and strikingly elevated ferritin levels, which are also commonly found in EVD, especially in the patients with a fatal outcome. In addition, less perforin mRNA expression was found in peripheral blood mononuclear cells of patients with fatal Ebola infection compared to those that survived (10), while animal studies indicated that CD8-mediated protection against Ebola is perforin-dependent (11).

Cytopenias are key laboratory markers of HLH and are attributed to severe cytokine-mediated inflammation (12). Patients with Ebola infection are often leucopenic at presentation, with abnormal low numbers of lymphocytes and an increased percentage of granulocytes (13). Interestingly, total leukocyte numbers abnormally increase with a rise in immature granulocytes and atypical lymphocytes while the disease develops: this may mirror hyperactivation of bone marrow by cytokines of the IL-1 family that have been proposed to mediate the systemic hyperinflammatory reaction in HLH (14). Thrombocytopenia is a constant feature of EVD, with bone-marrow studies suggesting that thrombocytopenia is not due to reduced platelet production but rather to diffuse intravascular coagulation, as has also been reported in 40–90% of HLH cases (12).

A striking recent finding is the hyperferritinemia in Ebola patients, with mean values around 10,000 μg/L in non-survivors, and 2,500 μg/L in non-fatal cases at day 6–10 after symptom onset. Although hyperferritinemia can be encountered in infections, autoimmune diseases, and several rare syndromes, such very high ferritin concentrations of more than 10,000 μg/L indicate HLH with a sensitivity of 90% and specificity of 96% (ref).

There are also characteristics of EVD that might argue against a classical HLH syndrome. Histiocytic infiltrates of various organs, including liver and spleen, are to be expected in HLH; however, a recent review of human pathology studies indicates that only minimal inflammation is observed in infected tissue and organs in fatal cases, despite high viral loads and necrotic lesions (5). However, Kupffer cell hyperplasia is seen in Ebola infections, similar to HLH (15). Furthermore, no hemophagocytosis has been reported to date in EVD, although very few (if any) studies have been done in this respect. Moreover, the hemophagocytic activity might not occur at all stages of the disease (12) and therefore does not have to be present to diagnose HLH (8).

CONCLUSION AND FUTURE PERSPECTIVES

In conclusion, the clinical manifestations and pathophysiology of severe EVD lead us to propose that HLH characterized by overwhelming macrophage activation is a key event in the pathogenesis of the Ebola infection.

It has been proposed that sepsis, systemic inflammatory response syndrome, multiple organ dysfunction syndrome, and HLH form a disease continuum that share a common mechanism: systemic immune dysregulation triggered by a specific external agent (12). We think that these conditions occur in Ebola virus infection. The metabolic and immune imbalance during EVD requires both optimal support

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measures and the removal of the trigger (infection) that stimulate inflammation. Unfortunately, the latter is not feasible, because no antiviral medication has been of proven efficacy in EVD.

The identification of HLH as part of severe EVD, however, brings the possibility for a pathophysiologically targeted approach of therapy, using the arsenal of immunomodulatory medication known to be successful in HLH. While plasma exchange, corticosteroids, and even immunosuppressive therapy have been proposed for treatment of HLH, special promise is represented by cytokine-directed therapy in the form of recombinant interleukin-1 receptor antagonist (anakinra). Treatment with anakinra has the advantage to interrupt the deleterious IL-1-mediated hyperinflammatory loop in macrophage activation syndrome, and it has been shown to be associated with remarkable effectiveness (16–20). Moreover, the excellent safety record of anakinra argues for its compassionate use in patients with severe EVD in humans.

AUTHOR CONTRIBUTIONS

All authors contributed substantially to the present work, revised the work critically, approved the final paper, and agreed to be accountable for all aspects of the work.

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MAJOR ARTICLE

Ebola Hemorrhagic Fever: Novel Biomarker Correlates of Clinical Outcome

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Background. Ebola hemorrhagic fever (EHF) outbreaks occur sporadically in Africa and result in high rates of death. The 2000–2001 outbreak of Sudan virus–associated EHF in the Gulu district of Uganda led to 425 cases, of which 216 were laboratory confirmed, making it the largest EHF outbreak on record. Serum specimens from this outbreak had been preserved in liquid nitrogen from the time of collection and were available for analysis.

Methods. Available samples were tested using a series of multiplex assays to measure the concentrations of 55 biomarkers. The data were analyzed to identify statistically significant associations between the tested biomarkers and hemorrhagic manifestations, viremia, and/or death.

Results. Death, hemorrhage, and viremia were independently associated with elevated levels of several chemokines and cytokines. Death and hemorrhage were associated with elevated thrombomodulin and ferritin levels. Hemorrhage was also associated with elevated levels of soluble intracellular adhesion molecule. Viremia was independently associated with elevated levels of tissue factor and tissue plasminogen activator. Finally, samples from nonfatal cases had higher levels of sCD40L.

Conclusions. These novel associations provide a better understanding of EHF pathophysiology and a starting point for researching new potential targets for therapeutic interventions.

Keywords. Ebola virus; biomarkers; hemorrhage; Gulu; hemorrhagic fever virus.

Ebola hemorrhagic fever (EHF) was first identified in 1976 during an outbreak in the Democratic Republic of the Congo (formerly known as Zaire). Since then, 21 additional outbreaks among humans have been recorded in sub-Saharan Africa. The largest of these outbreaks took place in the Gulu district of Uganda in 2000–2001 and was caused by Sudan virus (SUDV). This outbreak resulted in 425 cases, 216 of which were laboratory confirmed, and a 53% overall case-fatality rate [1].

The genus *Ebolavirus* includes 5 different viruses: SUDV, Tai Forest virus (TAFV), Reston virus (RESTV), Ebola virus (EBOV), and Bundibugyo virus (BDBV). EHF caused by EBOV has the highest fatality (57%–90%), followed by SUDV (41%–65%) and BDBV (40%); to date, RESTV infection has been asymptomatic in humans, and TAFV has only been identified in 2 human infections, both of which were nonfatal [2–4]. Clinical EHF is characterized by abrupt onset of fever, fatigue, headache, myalgia, and gastrointestinal distress 3–13 days after exposure to virus [5]. Many patients develop hemorrhagic manifestations, which has led to the term "hemorrhagic fever."

The samples collected during the Gulu outbreak have already yielded extensive information about EHF pathophysiology, including the observation that aspartate aminotransferase (AST), D-dimer, blood urea nitrogen (BUN), and creatinine levels are higher than normal, while calcium and albumin levels are lower than normal in samples from fatal EHF cases [6]. Elevated levels of the cytokines interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 10 (IL-10), and macrophage inflammatory protein 1β (MIP-1β) were also associated with fatal outcomes [7]. Additionally, human leukocyte antigen B67 (HLA-B67), HLA-B15, and marked CD8

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lymphopenia were associated with fatal outcome, and HLA-B7 and HLA-B14 were associated with nonfatal outcome [8, 9].

The goal of our study was to better understand the pathophysiology of EHF by correlating hemorrhagic manifestations and death with specific biomarkers. We measured levels of markers of immune function, endothelial activation, and coagulation in available patient serum samples, to determine whether alterations in levels of these biomarkers were associated with hemorrhagic manifestations, viremia, or death.

METHODS

Study Design

During the 2000–2001 Gulu outbreak, an international response team, including Centers for Disease Control and Prevention (CDC) staff, responded with clinical and technical assistance. Serum samples were frozen in liquid nitrogen in the field and have been stored that way since the time of the outbreak. For our analyses, samples were chosen to represent sex ratios, hemorrhagic manifestations, and death rates consistent with those observed during the outbreak (Table 1). Specimens were prioritized for novel analyses; serum chemistry analyses were performed on samples of sufficient volume. All samples were inactivated by γ irradiation before use.

Institutional Review Board (IRB)

IRB approval was obtained before initiating the study, and an exemption was granted by the CDC Human Research Protection Office.

Luminex-Based Assays

The following assays were purchased from Affymetrix (Santa Clara, CA) and performed according to the manufacturer's instructions: a 26-plex assay for granulocyte macrophage colony stimulating factor (GM-CSF), GROα, interferon α2 (IFN-α2), IFN-β, IFN-γ, IL-10, interleukin 12p70 (IL-12p70), IL-12p40, interleukin 1α (IL-1α), IL-1β, IL-1 receptor antagonist (IL-1RA), interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 5 (IL-5), IL-6, IL-8, IFN-γ–inducible protein 10 (IP-10), monocyte chemoattractant protein 1 (MCP-1), macrophage colony-stimulating factor (MCSF), MIP-1α, MIP-1β, soluble CD40 ligand (sCD40L), soluble E-selectin (sE-selectin), soluble Fas ligand (sFasL), tumor necrosis factor α (TNF-α), and vascular endothelial growth factor A (VEGF-A); a 2-plex assay for D-dimer and tissue plasminogen activator (TPA); a 5-plex assay for plasminogen activator inhibitor-1 (PAI-1), serum amyloid antigen (SAA), regulated on activation, normal T-cell expressed and secreted (RANTES), soluble intracellular adhesion molecule 1 (sICAM-1), and soluble vascular cell adhesion molecule (sVCAM-1); and single-plex assays for C reactive protein (CRP) and fibrinogen. Assays for ferritin and cortisol were performed as single-plex assays according to manufacturer's instructions (Millipore, Billerica, MA). 2-plex assays were performed for tissue factor (TF) and thrombomodulin according to the manufacturer's instructions (Millipore). For samples with values outside the upper end of the standard curve, additional dilutions were made as necessary to obtain accurate values for all analytes.

Table 1. Patient Characteristics

Characteristic	This Study (n = 86)	Gulu Outbreak (n = 425)
Age, y, range	6–60	0.25–81
Sex		
Female	56 (65)	267 (63)
Male	30 (35)	158 (37)
Any hemorrhagic manifestation	31 (36)	128 (30)
Death	41 (48)	224 (53)

Data are no. (%) of patients, unless otherwise indicated. Gulu outbreak data are from Okware et al [1].

Enzyme-Linked Immunosorbent Assays (ELISAs)

Mannose-binding lectin (MBL; Hycult Biotech, Plymouth Meeting, PA) and total immunoglobulin G (IgG; eBioscience, San Diego, CA) ELISAs were performed according to the manufacturers' instructions. For samples with values outside the upper end of the standard curve, additional dilutions were made as necessary to obtain accurate values for all analytes.

Serum Chemistry Analyses

A Piccolo comprehensive metabolic reagent disc was run on the Piccolo xpress Chemistry Analyzer (Abaxis, Union City, CA) to determine serum chemistry values for alanine aminotransferase (ALT), albumin, AST, alkaline phosphatase, calcium, chloride, creatinine, glucose, potassium, sodium, total bilirubin, total CO_2, total protein, and BUN. Only 132 of the 187 samples had sufficient volume for serum chemistry analyses.

Human Immunodeficiency Virus (HIV) Testing

The Multispot HIV-1/HIV-2 Rapid Test (BioRad, Hercules, CA) was used to assess the HIV status of the patient cohort. Two patients (ages 7 and 19 years) did not have sufficient sample for analysis.

Viremia Analysis via Measurement of RNA Copy Number

Total RNA was isolated from serum using the MagMAX-96 Viral RNA Isolation Kit on the MagMAX Express-96 Magnetic Particle Processor (Ambion, Grand Island, NY). In parallel, serial dilutions of a normal human serum specimen inoculated with a known titer of SUDV was subjected to RNA isolation and used to generate a standard curve. Real-time polymerase chain reaction was then performed as previously described [10].

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Statistical Analysis

Data on sex, age, date of symptom onset, viremia levels, hemorrhagic manifestations, HIV status, patient survivability, and date of sample collection were available for the 86 patients included in our study. Only samples taken 0–15 days from symptom onset were analyzed, to represent the normal course of the illness.

Analysis of variance (ANOVA) was conducted for each of the 55 analytes, using sex, age, day from symptom onset, viremia, death, hemorrhaging, and HIV status as the independent variables. All variables were converted to categorical variables (meaning that there was limited number of outcomes [ie, viremic vs not viremic]) because of the number of samples and the distribution of the numerical data, with the date from symptom onset converted to 3 categories (0–5 days, 6–10 days, and 11–15 days). An initial overall α of 0.25 was set, and the Bonferroni inequality was used to arrive at a critical P value of .0045 for the individual analytes. Using these criteria, 29 of the 55 analytes had statistically significant differences. Knowing the impact of multiple testing on the power of tests, the technique of Yoav and Hochbergs was used to control the false discovery rate to balance the power and type 1 error of the analyses [11]; this correction resulted in 10 additional analytes meeting the criteria for statistical significance. The independent variables were analyzed to measure colinearity, but no significant variance inflation was observed.

Ultimately, our study included 161 samples, each described by 9 categorical variables. Each sample was analyzed for 41 different analytes (one sample was insufficient to perform a subsequent analysis of 2 analytes). An analysis of metabolic information was attempted for all samples, but only 132 samples had sufficient volume for analyzing 14 additional analytes.

Eight analytes (IL-12p70, IL-12p40, IL-2, IL-4, IL-5, sFasL, IFN-γ, and IFN-α2) had statistically significant correlations but were excluded from analysis because $\geq$30% of the samples had a value below the limit of detection. We therefore did not attempt to make conclusions based on data that could have been affected by degradation over time.

Model Selection

After the initial ANOVA, 27 significant analytes were selected for further analysis. Stepwise regression for each significant analyte was used to find the best model, excluding insignificant variables at an α of 0.05. Particular attention was paid to those analytes in which age, death, viremia, hemorrhaging, and HIV status were significant. Ten analytes were significant for age, 16 for death, 7 for viremia, 8 for hemorrhaging, and 2 for HIV status. We compiled the analytes that were significantly different for these variables and calculated the means and standard error for each interval.

Finally, 2 additional statistical tests were performed to measure the relationships between death and both viremia levels and the presence of hemorrhaging. The distribution of the viremia levels was clearly nonnormal, so the Mann–Whitney U test was used to determine differences in viremia for patients with fatal and those with nonfatal outcomes. Patients with no measurable viremia were excluded from analysis. The survivability of patients with and those without hemorrhaging was also examined, but no significant differences were found.

RESULTS

Study Population

The study included 86 EHF patients from the 2000–2001 Gulu outbreak that were representative of the outbreak as a whole. As noted in previous outbreaks, more women were affected than men, likely because women are commonly caretakers, a known risk factor for contracting EHF. Thirty-six percent of the patients in this study had hemorrhagic manifestations (defined as any of the following: vomiting blood, blood in the stool, or bleeding from the gums, skin, or eyes), and approximately half of the patients died, consistent with reported death rates for SUDV (Table 1). One limitation to our study was that the limited sample volume influenced our selection of samples because we only selected patients for whom there were sufficient sample volume to complete the desired testing. This may have biased our data slightly toward the nonfatal group.

In the filovirus field, it has generally been assumed that immune system deficiencies associated with HIV infection would likely negatively impact EHF outcome. As the HIV status of EHF patients has never been determined previously, HIV testing was conducted on patients included in this study. None of the patients <18 years of age were HIV positive. Of the 67 adults (ages 18–60 years), 12 (17.9%) were HIV positive. This rate is roughly consistent with data on HIV prevalence in the Gulu region of Uganda during the time of the outbreak; 12% of women attending antenatal clinics were reported to be HIV positive [12]. Of the 12 HIV-positive adults, 5 were male and 7 were female. Although $CD4^+$ T-cell counts were unknown in these patients, 50% of the HIV-positive adults (3 males and 3 females) had fatal outcomes. This mortality rate was approximately the same as that in the total adult population, suggesting that an individual's HIV status did not significantly affect outcome during SUDV infection.

Study Design

Fifty-five different biomarkers were assessed in 187 serum samples in this study. Thirty-six patients had $\geq$2 serial samples collected 1–15 days after onset of symptoms; composite data rather than individual patient data are presented, to provide the most objective data for analysis. Supplementary Table 1 provides the statistical significance for each biomarker tested and its relationship to the examined variables. The data presented in this article represent the statistically significant associations observed

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Table 2. Coefficient of Variance (CV) Between Repeat Chemistry Measurements After 12 Years of Storage in Liquid Nitrogen

CV	ALT	AST	BUN	CRE	TP	ALB
Percentage difference, mean (range)	2.3 (0–7.4)	1.5 (0–4.4)	1.7 (0–7.1)	2.2 (0–5.4)	0.96 (0–2.9)	0.75 (0–2.8)

Abbreviations: ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CRE, creatinine; TP, total protein.

between a given biomarker and death, hemorrhagic manifestations, or viremia.

Serum Chemistry Analysis and Viremia

Serum chemistry analyses were performed on many patients as part of their clinical management during the 2000–2001 outbreak. These data were still available, allowing for a direct assessment of the stability of several metabolic analytes after 12 years of storage in liquid nitrogen. Many analytes were strikingly stable, as shown by a maximal coefficient of variance of 7.4% between the original measurement and the repeat measurement for ALT, AST, BUN, creatinine, total protein, and albumin levels (Table 2). The more labile components, CO_2 and electrolytes, were excluded from our analysis.

Previously reported associations between fatal outcomes and AST, BUN, creatinine, and albumin levels [6] were again seen in our study (data not shown) [6]. Higher levels of viremia were associated with a fatal outcome, as previously demonstrated [10] (Figure 1).

Cytokines and Chemokines

Cytokines and chemokines are a diverse, extensively studied group of proteins that modulate the immune response. We analyzed 25 different cytokines and chemokines. IL-1α, IL-1RA, IL-6, MCP-1, MCSF, and MIP-1α levels were all elevated in patients with a fatal outcome (Figure 2*A*–*E* and 2*G*).

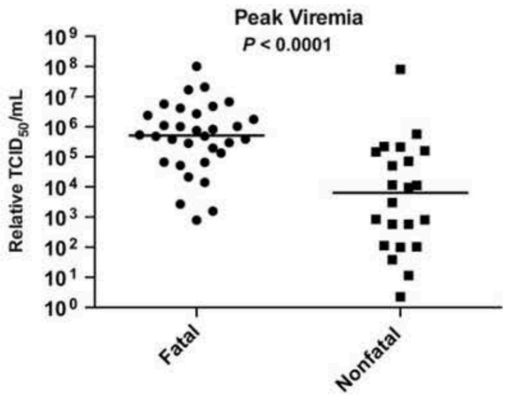

Figure 1. Viremia as a function of clinical outcome. Patients with fatal outcomes had higher viral loads. $TCID_{50}$, median tissue-culture infective dose.

Previously published data have shown associations between MIP-1β, IL-6, IL-8, and IL-10 levels and fatal outcomes in SUDV-infected patients [7]. All of these prior associations were observed in our study. However, IL-8 levels were only elevated very late in the infection in patients with a fatal outcome (data not shown); MIP-1β levels tended to be higher in patients with fatal outcomes but did not meet the cutoff for statistical significance; and IL-10 levels demonstrated an age-specific association with death. GRO-α levels, similar to IL-8 levels, were only elevated late during infection in patients who died (data not shown). IL-1α, MCSF, and IP-10 levels were elevated in samples with documented viremia, compared with samples with no detectable virus, but this finding was independent of their relationship with death (data not shown). MCSF, MIP-1α, and IP-10 levels were also elevated in patients who developed hemorrhagic manifestations (Figure 2*F*, 2*H*, and 2*I*). Unexpectedly, elevated levels of sCD40L correlated with nonfatal outcomes, a first for any report of EHF (Figure 2*J*).

Biomarkers of Inflammation

The acute phase response refers to a constellation of host responses that occur following infection and in other inflammatory diseases [13]. These responses are classically triggered by proinflammatory cytokines, such as IL-6, IL-1, IFN-γ, and TNF-α. Increased liver production of CRP and SAA and decreased production of albumin are common consequences of the acute-phase reaction. In addition, hypergammaglobulinemia and hyperferritinemia are often seen. These markers of inflammation are used clinically in many disease processes to help in diagnosis and to follow a patient's response to therapy. CRP levels were within the normal range in all samples and were excluded from further analysis. Ferritin levels were elevated in patients with fatal outcomes and in those with hemorrhagic manifestations (Figure 3*A* and 3*B*). Ferritin levels were also associated with viremia, independent of the associations with death and hemorrhage (data not shown). Ferritin levels were higher in HIV-positive patients than in HIV-negative patients (data not shown); this has been previously noted specifically in association with advanced HIV disease [14, 15]. HIV-positive patients also had higher total IgG levels than HIV-negative patients (data not shown), a well-documented phenomenon [16]. Finally, levels of sICAM, a marker of endothelial activation [17], were elevated in patients with hemorrhagic manifestations (Figure 3*C*)

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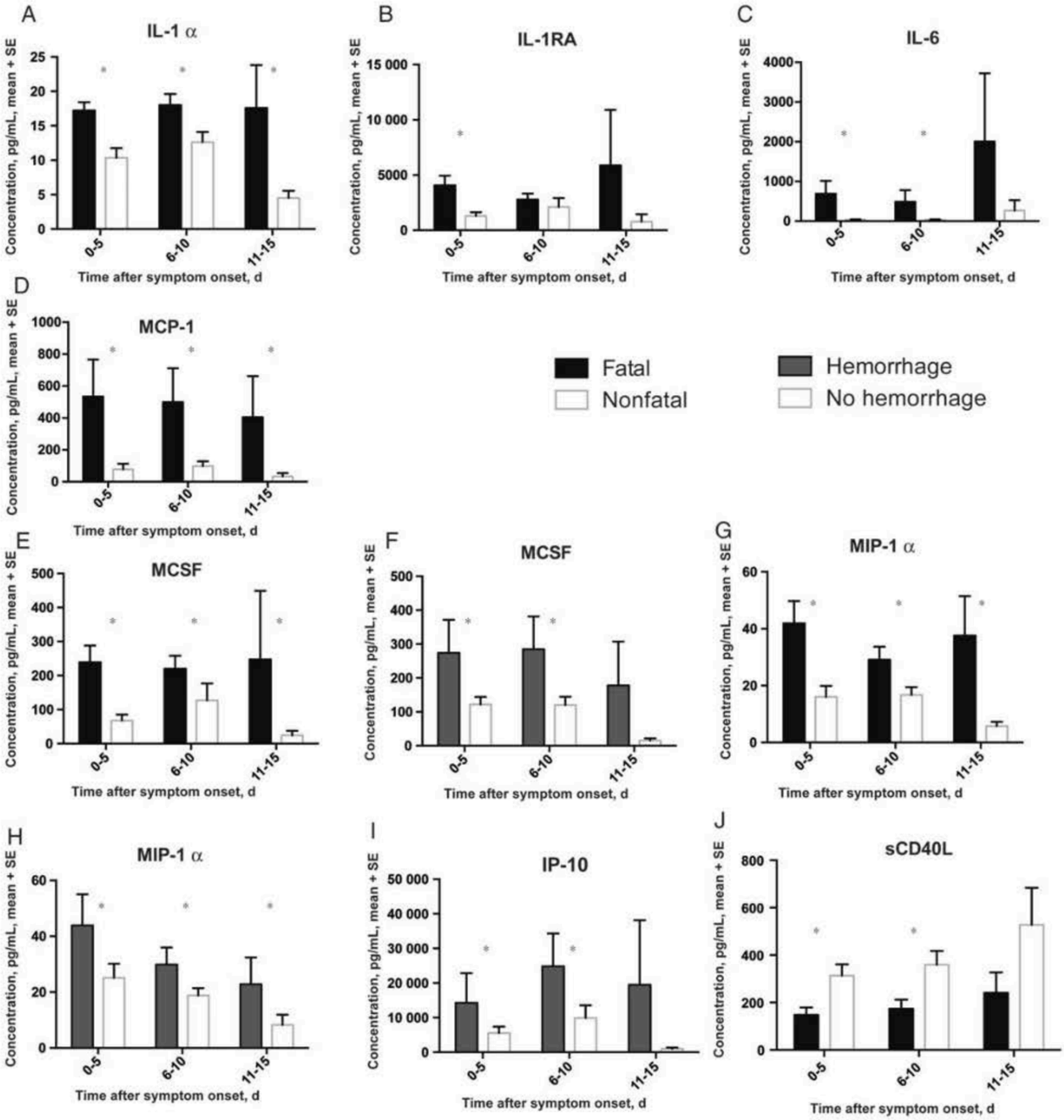

Figure 2. Cytokines and chemokines associated with a fatal outcome (black bars) or hemorrhagic manifestations (red bars). *$P \leq .05$. Abbreviations: IL-1α, interleukin 1α; IL-1RA, interleukin 1 receptor antagonist; IL-6, interleukin 6; IP-10, interferon γ–inducible protein 10; MCP-1, monocyte chemoattractant protein 1; MCSF, macrophage colony-stimulating factor; sCD40L, soluble CD40 ligand; SE, standard error.

Biomarkers of Coagulopathy

The frequency of hemorrhagic manifestations during EHF warranted an examination of the measureable factors that control hemostasis. The delicate balance of coagulation and fibrinolysis is depicted in a simplified model in Figure 4. In this study, PAI-1, fibrinogen, TPA, D-dimer, thrombomodulin, and TF levels were measured in all patient samples. TPA, D-dimer, thrombomodulin, and TF levels were all statistically significant in our analysis. Thrombomodulin levels were elevated in patients with fatal outcomes and in patients with hemorrhagic manifestations (Figure 4*A* and 4*B*). D-dimer levels were elevated in patients with a fatal outcome (Figure 4*C*), as previously reported [6]. TPA levels were higher in viremic patients (Figure 4*D*). TF levels were within the normal range in all samples

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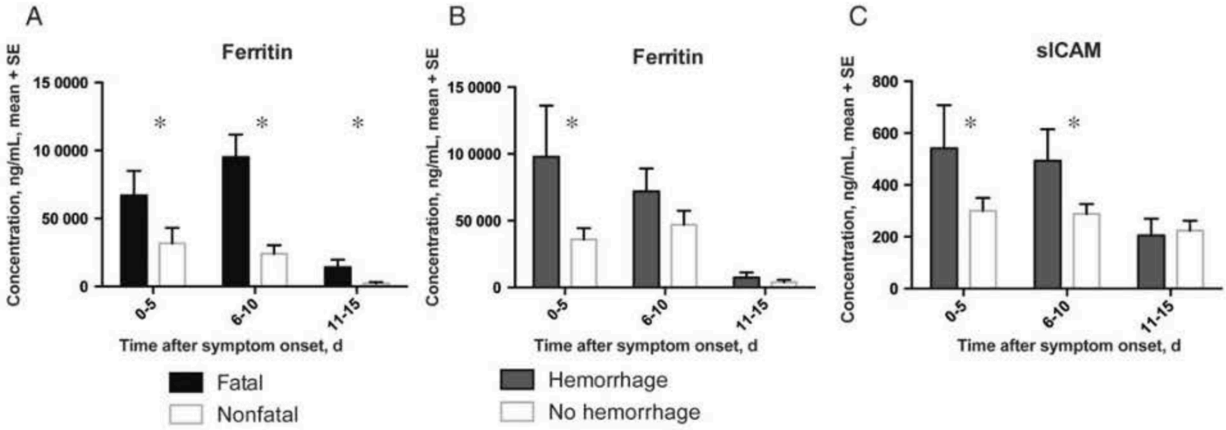

Figure 3. Markers of inflammation associated with a fatal outcome (black bars) or hemorrhagic manifestations (red bars). *$P \leq .05$. Abbreviations: SE, standard error; sICAM, soluble intracellular adhesion molecule 1.

tested but were slightly higher in samples with detectable viremia than in those without detectable viremia (Figure 4*E*). Notably, TF levels were not associated with hemorrhagic manifestations or death.

DISCUSSION

We found associations between multiple proinflammatory cytokines and increased death rates, as previously shown in humans [7, 18–20] and EBOV-infected nonhuman primates [21], consistent with the idea of systemic inflammation contributing to a fatal outcome. We also found several novel associations, most strikingly, the association between increased sCD40L levels and nonfatal outcomes. Since sCD40L is largely produced by activated platelets [22], increased sCD40L levels could represent ongoing repair of altered endothelium by activated platelets. Additionally, nitric oxide (NO) inhibits platelet activation [23], and elevated NO levels have been reported in fatal EHF cases [9]. Therefore, the deleterious effects of NO could be related to inhibiting platelet activation, in addition to its vasodilatory effects. Effective repair of altered endothelium by activated platelets could lead to improved hemodynamic stability in patients and, thus, improved clinical outcomes.

Ferritin, another biomarker we examined, is a well-established acute-phase reactant that has been associated with severe disease in both dengue and Crimean Congo hemorrhagic fevers [24, 25]. Ferritin is the intracellular storage form of iron, and its levels increase during times of stress or inflammation. Ferritin levels were higher in samples from patients with EHF who had fatal outcomes, detectable viremia, and hemorrhagic manifestations; thus, ferritin could prove to be a useful clinical maker of EHF severity.

The striking clinical manifestation of hemorrhage during historic EHF outbreaks led to the naming of the disease as Ebola *hemorrhagic* fever, which has resulted in the perception that hemorrhage occurs in most patients and is associated with fatal outcomes. However, published data from the 3 largest EHF outbreaks, caused by EBOV (Kikwit, Democratic Republic of the Congo, 1995), SUDV (Gulu, Uganda, 2000), and BDBV (Bundibugyo, Uganda, 2007), demonstrate hemorrhagic manifestations in only 41%, 30%, and 46.5% of total cases, respectively [1, 3, 26]. Furthermore, no significant correlation between hemorrhage and death has been found in these same outbreaks of EBOV and BDBV infections. Consistently, we found no statistically significant differences in survival between patients with and those without hemorrhage ($P = .23$). Therefore, it is not surprising that, of the 6 different coagulation and fibrinolysis markers we assessed, only thrombomodulin was associated with both fatal outcome and hemorrhagic manifestations. Thrombomodulin is a marker of endothelial activation/dysfunction and, as discussed below, has many functions besides inhibiting coagulation. There is a common perception, not only in EHF but also in routine sepsis, that correction of coagulopathy will improve survival outcomes. However, therapeutic interventions that have focused on targeting coagulopathy in nonhuman primate models of EBOV only moderately increased survival [27, 28], and use of activated protein C in septic patients in the intensive care unit has been similarly disappointing [29]. A large focus has been placed on understanding the role of TF in EHF, given the marked increases in TF expression seen in monocytes in nonhuman primates infected with EBOV [30]. However, when TF levels were examined in patients from this outbreak, which was caused by SUDV, none of the patients had elevated levels of TF outside of the normal range, and there were no associations between TF levels and death or hemorrhage. These data are limited by our ability to only sample plasma TF versus monocyte-associated TF, although plasma and monocyte TF levels correlated in a human study of

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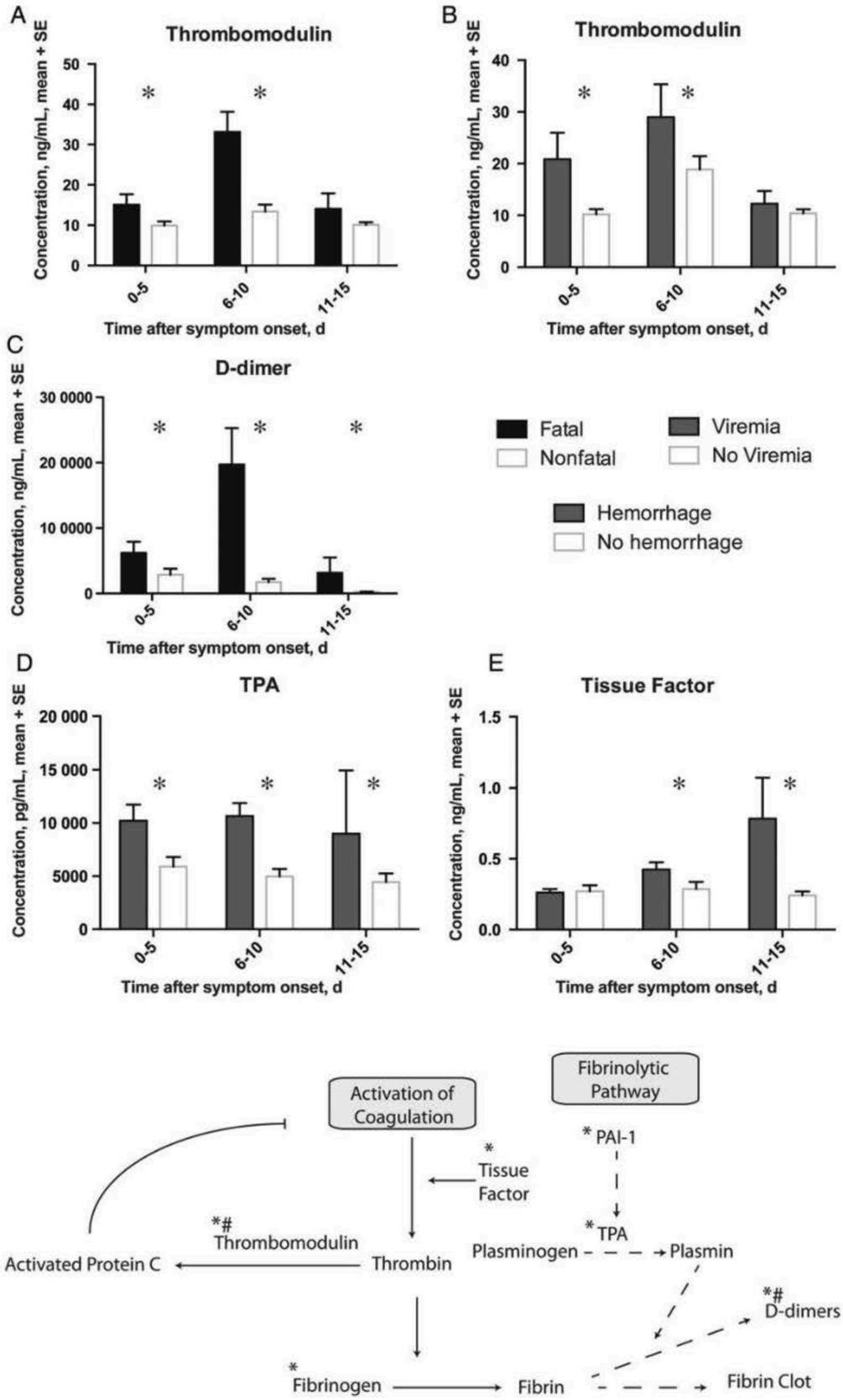

Figure 4. Markers of coagulation and fibrinolytic pathways associated with a fatal outcome (black bars), hemorrhagic manifestations (red bars) or viremia (blue bars). *$P \leq .05$. A simplified model of the coagulation/fibrinolytic pathway is also depicted. Proteins that are known to play a major role in the pathways are noted. Arrows indicate activation while bars indicate inhibition. *, analytes assessed in the study; #, analytes associated with death.

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thrombosis [31]. Finally, the nonhuman primate studies were performed with EBOV, whereas the patients in this study were infected with SUDV, so these TF differences could be virus or host related.

Thrombomodulin is released by an activated endothelium and functions as an anticoagulant by activating protein C when present in the microenvironment on the surface of endothelial cells. In our study, thrombomodulin levels were elevated in samples from patients who died and from those with hemorrhagic manifestations, the latter being consistent with its anticoagulant function. Thrombomodulin has several protein domains that are responsible for its anticoagulant activity, but it also contains a C-type lectin domain that has antiinflammatory activities, including maintaining endothelial integrity [32]. The fact that EBOV GP protein binds to other C-type lectins [33–35] makes the thrombomodulin C-lectin domain an attractive subject for further study. If thrombomodulin's C-lectin domain–mediated functions are inhibited by association with viral GP, this could provide a possible explanation for how these viruses are able to disrupt endothelial function without causing direct cytopathic effects. Alternatively, overactivation of endothelial cells, leading to shedding of thrombomodulin into the plasma, could result in a loss of thrombomodulin's stabilizing function at endothelial junctions, contributing to vascular leakage.

MCSF, MIP-1α, IP-10, sICAM, and thrombomodulin levels were all associated with hemorrhage in our study, and on the basis of findings in the literature, their activities can all be correlated with leukocyte/endothelium interactions. In one possible model, these cytokines could recruit leukocytes to areas of inflammation, and production of adhesion molecules such as ICAM would facilitate leukocyte adhesion, rolling, and diapedesis. Activated endothelial cells would then release thrombomodulin into the bloodstream, so it would no longer be present in the microenvironment to activate protein C and coagulation, nor would it be present to stabilize the endothelium. This would leave an activated, leukocyte-enriched, procoagulant endothelium, causing dysregulated hemostasis, which could manifest clinically as hemorrhage.

Future pathogenesis models could evaluate the possible role of activated platelets in improving clinical outcomes by therapeutic administration of platelets, inhibitors of NO synthetase, or active sCD40L to infected animals. Finally, the potential roles of thrombomodulin C-lectin domains in modulating EHF outcomes should be further studied at the molecular level to determine whether viral glycoproteins interact with thrombomodulin. If this proves to be the case, evaluating the role of thrombomodulin C-lectin domains in animal models would be warranted.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgment. We thank Tatyana Klimova, PhD, for editing the manuscript.

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Potential conflict of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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From: Agnes Binagwaho
Sent: Sat, 12 May 2018 06:38:24 +0200
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Sullivan, Kevin;Barbara Bush (b)(6) (b)(6) Mark Dybul;Yvette Umwali (b)(6) Prince, Emma;Walker, Annie
Subject: Re: The Lazarus Effect: 15 Years Later Video

Dear Sully and Team,
It was a pleasure and a privilege to be part of this fantastic and well organized event and to have contributed to pay tribute to a modest President who deserve it.
By meeting my friends I left Dallas 100% energized!
Thank you for all this.
Warm regards
Agnes

On Thu, May 10, 2018 at 10:00 PM, Fauci, Anthony (NIH/NIAID) [E] (b)(6) wrote:
Sully:
You and your team did a spectacular job in putting together and implementing the program. It was a wonderful experience and I felt privileged to be included in this incredible group. Many thanks.
Best regards,
Tony

-----Original Message-----
From: Sullivan, Kevin (b)(6)
Sent: Thursday, May 10, 2018 12:47 PM
To: Barbara Bush (b)(6) Fauci, Anthony (NIH/NIAID) [E] (b)(6) (b)(6) (b)(6) (b)(6) (b)(6) (b)(6) (b)(6) Mark Dybul (b)(6) Yvette Umwali (b)(6) (b)(6)
Cc: Prince, Emma (b)(6) Walker, Annie (b)(6)
Subject: The Lazarus Effect: 15 Years Later Video

Good morning all,

On behalf of Emma, Annie and our Events team, thank you so much for all you did to make last night such a memorable event. We have gotten terrific feedback - there is no question our audience was inspired about what's been accomplished...and understands the importance of keeping PEPFAR funded and strong.

Here is a link to the video from bushcenter.org. Feel free to share freely:

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(b)(6)

Please don't hesitate to reach out if I can be helpful on this end.

Sincerely,

Sully

Kevin Sullivan
Senior Advisor, External Affairs
George W. Bush Presidential Center

T (b)(6) F 214.855.4347 M (b)(6)
(b)(6)

--

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From: Eisinger, Robert (NIH/NIAID) [E]
Sent: Fri, 11 May 2018 22:04:26 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: FW: Lancet Commission - request for review
Attachments: Main Manuscript_051018 rev BE LD.docx

FYI

From: Eisinger, Robert (NIH/NIAID) [E]
Sent: Friday, May 11, 2018 6:04 PM
To: 'Mike Reid' (b)(6)
Cc: (b)(6) (b)(6) Doepel, Laurie (NIH/NIAID) [E]
(b)(6)
Subject: RE: Lancet Commission - request for review

Mike and David

Thank you for the opportunity to review the latest version of the Lancet Commission Report on TB. Please find in the attachment Laurie Doepel's and my proposed modifications to the main manuscript. Laurie will send our combined comments on the Executive Summary tomorrow. Congratulations on an excellent document, we know this has been quite a challenge.

If we can provide any additional information or assistance, please let us know.

Thank you.
Bob

From: Mike Reid (b)(6)
Sent: Thursday, May 10, 2018 3:00 PM
To: Eric Goosby (b)(6)
Cc: David McKey (b)(6)
Subject: Lancet Commission - request for review

Dear Colleagues,

Thanks to all of you for contributing some portion of The Lancet Commission on TB report, 'Building a TB free world.'

I am attaching a close-to-final draft of the report* and the executive summary for all of you to review. This draft has been reviewed by the Commissioners, and as such, the recommendations included here reflect their consensus. The full report, as well as additional panels/boxes [many of which you contributed to!] are available at the links below:

Box Users:
Report Sections

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Tables/Figures
Panels/Appendices
Track change version of Report Sections

Dropbox Users:
Report Sections
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Panels/Appendices
Track change version of Report Sections

As contributing authors, I invite you to review and welcome comments on the draft. In the interests of time, and to ensure that we have the best possible chance of getting this report published in time for/at the time of the High Level Meeting in September, please send any substantive comments by **May 13.** I understand that this is a very, very narrow turnaround time (apologies); an inevitable consequence of the writing process and the looming deadline. However, please do me and Eric know if you have any concerns or questions about the draft.

Writing by committee also means that some of your original contributions may have morphed and/or messages changed with input from Commissioners. Nonetheless, to reiterate my opening statement, your contribution to this effort is greatly appreciated.

Finally, please check the title page attached [and confirm your affiliations are correct, etc]. Kindly, cc David McKey in your response since he is helping to ensure that all of these details are inputted correctly. I will follow up shortly with a request to complete a conflict of interest form to accompany the manuscript submission.

Thanks and kind regards,

Mike

Lancet Commission Secretariat

Assistant Professor
Global Health Delivery & Diplomacy
Global Health Sciences | UCSF
550 16th Street, 3rd Floor
San Francisco, California 94158
Cell: (b)(6)
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Pager: (b)(6)
Alt Email: (b)(6)

http://profiles.ucsf.edu/michael.j.reid

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*There are some analyses outstanding. The Lancet have agreed to review even without these included.

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Fri, 11 May 2018 21:32:57 +0000
To: Marston, Hilary (NIH/NIAID) [E]
Cc: Fauci, Anthony (NIH/NIAID) [E];Lane, Cliff (NIH/NIAID) [E]
Subject: RE:

From: Marston, Hilary (NIH/NIAID) [E]
Sent: Friday, May 11, 2018 5:26 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject:

Dr. Fauci asked me to reach out to you about the mAb114 request from the DRC, on his behalf (and Cliff's). NIAID would like to be supportive of the country's research response plans and the WHO coordination function. DRC has a lead person for the outbreak response (including the research response)who interacts with the WHO. To that end, we would recommend that you ask your INRB contact to identify that DRC lead person and let *them* know about the INRB interest in using mAb114. Assuming the DRC MOH is supportive, the DRC lead should coordinate with the WHO (Anna Maria Henao-Restrupo) where by the official research agenda is led by the DRC in partnership with the WHO. We can then follow their lead, under the auspices of the NIAID-WHO MOU.

Hilary Marston, MD, MPH
Medical Officer and Policy Advisor for Global Health
NIAID Office of the Director
(b)(6)
(b)(6) (cell)

AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Fri, 11 May 2018 17:30:33 +0000
To: Fauci, Anthony (NIH/NIAID) [E];Schwetz, Tara (NIH/NIAID) [E]
Subject: discuss the HIV/AIDS perspective w Tara
Attachments: Re: Request: Time on ASF's Schedule, Materials: HIV/AIDS Perspective Meeting on 5/18

AMERICAN OVERSIGHT

From: Schwetz, Tara (NIH/NIAID) [E]
Sent: Fri, 11 May 2018 17:29:17 +0000
To: Vance, Meaghan (NIH/NIAID) [C]
Cc: Conrad, Patricia (NIH/NIAID) [E]
Subject: Re: Request: Time on ASF's Schedule

2:30 pm will work well. Thanks!

Best,

Tara A. Schwetz, PhD
Branch Chief, SPEB/NIAID
A: Building 31, Room 7A46D
P: (b)(6) | **M:** (b)(6)

From: "Vance, Meaghan (NIH/NIAID) [C]" (b)(6)
Date: Friday, May 11, 2018 at 12:28 PM
To: Tara Schwetz (b)(6)
Cc: "Conrad, Patricia (NIH/NIAID) [E]" (b)(6)
Subject: RE: Request: Time on ASF's Schedule

Sure. We can Friday, May 18th at 11 or 2:30pm.

From: Schwetz, Tara (NIH/NIAID) [E]
Sent: Friday, May 11, 2018 11:29 AM
To: Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Cc: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: Request: Time on ASF's Schedule

Meaghan,

Would it be possible to get some time on ASF's schedule next week to discuss the HIV/AIDS perspective? Thanks so much!

Best,

Tara A. Schwetz, PhD
Branch Chief, SPEB/NIAID
A: Building 31, Room 7A46D
P: (b)(6) | **M:** (b)(6)

AMERICAN OVERSIGHT

From: Schwetz, Tara (NIH/NIAID) [E]
Sent: Mon, 14 May 2018 17:40:29 +0000
To: Vance, Meaghan (NIH/NIAID) [C]
Cc: Conrad, Patricia (NIH/NIAID) [E]
Subject: Materials: HIV/AIDS Perspective Meeting on 5/18
Attachments: 2018-5-03_HIV Perspective_v2 rev .docx

Meaghan,

Please find attached a draft of the perspective in preparation for the meeting on Friday afternoon. Do let me know if anything else is needed.

Best,

Tara A. Schwetz, PhD
Branch Chief, SPEB/NIAID
A: Building 31, Room 7A46D
P: (b)(6) | **M:** (b)(6)

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From: Marston, Hilary (NIH/NIAID) [E]
Sent: Fri, 11 May 2018 21:26:26 +0000
To: Fauci, Anthony (NIH/NIAID) [E]

Dr. Fauci asked me to reach out about the mAb114 request from the DRC, on his behalf (and Cliff's). We would all (VRC included) like to be supportive of the country's research response plans and the WHO coordination work. To that end, we would recommend that you ask your INRB contact to identify the DRC lead for the outbreak response (including the research response) and let *them* know about the interest in using mAb114. Assuming MOH leads are supportive, It should then be on the official research agenda being led by the DRC in partnership with the WHO. We can then follow their lead, under the auspices of the NIAID-WHO MOU.

Hilary Marston, MD, MPH
Medical Officer and Policy Advisor for Global Health
NIAID Office of the Director
(b)(6)
(b)(6) (cell)

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Fri, 11 May 2018 21:02:10 +0000
To: (b)(6) Davey, Richard (NIH/NIAID) [E];Chertow, Daniel (NIH/CC/CCMD) [E]
Subject: FW: Ebola and MAS
Attachments: Anakinra for Ebola proposal 2018.pdf, Interleukin_1_Receptor_Blockade_Is_Associated_With.5.pdf, Ebola biomarkers 2014.pdf, J Rheumatol-EBOLA editorial 2015[2].pdf, Ebola MAS Frontiers in Medicine 2015[2].pdf, Ebola and ferritin[2].pdf

Cliff/Rick/Dan:
Do you have a quick opinion on this?
Thanks,
Tony

From: Bluestone, Jeffrey (Private) (b)(6)
Sent: Friday, May 11, 2018 4:14 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: Ebola and MAS

Tony, you may remember that the last time there was a big Ebola outbreak, I forwarded a suggestion made by an former grad student/post-doc of mine, Randy Cron, who is currently Chair of Pediatric Rheumatology at UAB. He had hypothesized that much of the morbidity of Ebola was due to an MAS syndrome and suggested that it would be worth trying Anakinra (the il-1 antagonist) in a few patients. I think at the time, you had suggested he continue to study and perhaps put in a grant. Since that time he and others have continued to study the similarities between Ebola morbidity and MAS. He remains convinced that there would be great value. I met with Hugh yesterday and he told me how involved you are in these outbreaks and the commitment you having including travel to Africa and direct involvement in the treatment of patients coming back to US. I also know that Cliff Lane is very involved. I am attaching a letter from Randy and some papers with the hope you or your colleagues involved in this new outbreak might open a dialogue and see if such a treatment is worth trying.

Jeff

==========
Jeffrey A. Bluestone, Ph.D.
A.W. and Mary Margaret Clausen Distinguished Professor
Director, Hormone Research Institute
University of California, San Francisco 415-514-0417
HSW 1112 Box 0540, 513 Parnassus Ave, San Francisco, CA 94143-0540
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UAB MEDICINE
PEDIATRIC RHEUMATOLOGY

Children's of Alabama

May 11, 2018

Anthony Fauci, MD
Director, NIAID

Dear Dr. Fauci:

As you are aware, Ebola is a rapidly fatal viral infection that has returned to W. Africa in recent days [https://www.cnn.com/2018/05/10/health/drc-confirms-first-ebola-death/index.html]. At present, there are no known effective therapies, and the toll from this current Ebola outbreak may potentially reach millions of people.

A study from the Centers for Disease Control (CDC) detailing the 2000-2001 Ebola outbreak noted that elevated serum ferritin levels were strongly associated with both fatal outcomes and hemorrhagic features of disease (1). Depending on the cut-off value, elevated serum ferritin can be both a sensitive and specific marker of cytokine storms syndromes, such as macrophage activation syndrome (MAS) and hemophagocytic lymphohistiocytosis (HLH) (2). HLH/MAS is a frequently fatal complication of infectious, oncologic, and rheumatic conditions that results from a pro-inflammatory cytokine storm (3). The clinical, laboratory, and pathologic features of HLH/MAS are strikingly similar to Ebola and other forms of hemorrhagic fever, including high fever, elevated serum ferritin values, liver dysfunction, pancytopenia, hemophagocytosis, central nervous system dysfunction, hemorrhages, etc. (3).

HLH and hemophagocytosis have already been associated with other hemorrhagic fever viruses, including Crimean-Congo (4) and Dengue (5). Indeed, elevated serum ferritin levels have been reported in Dengue (6) and associated with the severity of HLH manifestations in Crimean-Congo fever (7). It is, therefore, not much of a stretch to consider Ebola hemorrhagic fever as a likely trigger of HLH/MAS.

Familial HLH with homozygous defects in lymphocyte cytolytic pathway genes is uniformly fatal unless treated with an aggressive etoposide-based protocol (HLH-2004) followed by bone marrow transplantation (3). Secondary HLH or MAS presenting beyond infancy is often treated with immunosuppressive therapy such as high dose corticosteroids and cyclosporine A (8). Indeed, immunosuppression has successfully treated Dengue (9). Recently, targeted cytokine inhibition has been shown to successfully treat HLH/MAS (10). In particular, blockade with the drug anakinra, a recombinant human IL-1 receptor antagonist (IL-1Ra), has been successfully used to treat refractory cases of MAS (11). Moreover, anakinra was safe and doubled the survival of patients with sepsis (12). Experimentally, IL-1Ra deficient mice were shown to have enhanced susceptibility to Ebola virus infection (13). Finally, we are currently conducting a randomized, double-blinded placebo controlled trial of anakinra in treating non-oncologic associated MAS [NCT02780583].

Pediatric Rheumatology, Children's of Alabama
Children's Park Place North, 1601 4th Avenue South Birmingham, Alabama 35233
Mailing Address: CPPN, Suite G10, 1600 7th Avenue South, Birmingham, Alabama 35233-1711
Tel 205.638.9438 fax 205.638.2875 www.uab.edu/medicine/peds/rheum

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The benefits of anakinra specifically are the following:

1) It is quick acting. Within 24-72 hours, a clinical and laboratory response will be evident (14).
2) It is remarkably safe in humans (>10,000 received) as a recombinant human protein (15).
3) It has a very short half-life (4-6 hours), so it is rapidly cleared should safety concerns arise (15).
4) It is easily administered intravenously (IV), or subcutaneously when IV access is limited (16).
5) It has a large therapeutic window with safe dosing in humans ranging from 1 to 48 mg/kg/day (17).
6) It binds both interleukin 1 alpha and beta (IL-1α and IL-1β) (15).

For the current outbreak of Ebola in W. Africa, we, therefore, propose to administer anakinra (100 mg IV/SC q 6 hours) (18) early in the course of disease in individuals with a clinical diagnosis of Ebola hemorrhagic fever. The ability to administer anakinra subcutaneously bypasses the necessity of IV access which proved difficult in the last large Ebola outbreak in W. Africa. We suggest monitoring fever, overall clinical well-being, and any or all of the following labs if available: AST, ALT, D-dimers, platelet count, CRP, and ferritin. Responses should be evident within 24-72 hours of administration with very little risk, infectious or otherwise, but a large potential rapid benefit to the patient. Anakinra serves as a powerful anti-pyretic if nothing else. As we and others have suggested previously (19, 20), we hope you consider this relatively simple to administer, quick acting, and remarkably safe agent in treating those suffering from Ebola.

Happy to discuss by phone at your convenience.

Randy Q. Cron, MD, PhD
Professor of Pediatrics & Medicine
Arthritis Foundation, Alabama Chapter Endowed Chair
Director, Division of Pediatric Rheumatology
Children's Hospital of Alabama/
University of Alabama at Birmingham
Children's Park Place North, Ste. G10
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AMERICAN OVERSIGHT

Interleukin-1 Receptor Blockade Is Associated With Reduced Mortality in Sepsis Patients With Features of Macrophage Activation Syndrome: Reanalysis of a Prior Phase III Trial*

Bita Shakoory, MD[1]; Joseph A. Carcillo, MD[2]; W. Winn Chatham, MD[3]; Richard L. Amdur, PhD[4]; Huaqing Zhao, PhD[4]; Charles A. Dinarello, MD[5]; Randall Q. Cron, MD, PhD[6]; Steven M. Opal, MD[7]

*See also p. 443.

[1]Division of Rheumatology, Department of Medicine, George Washington University, Washington, DC.

[2]Department of Critical Care and Pediatrics, University of Pittsburgh Medical Center, Pittsburgh, PA.

[3]Division of Clinical Immunology and Rheumatology, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL.

[4]Department of Clinical Research, Temple University, Philadelphia, PA.

[5]Division of Infectious Diseases, Department of Medicine, University of Colorado Denver, Aurora, CO.

[6]University of Alabama at Birmingham, Division of Pediatric Rheumatology, Department of Pediatrics, University of Alabama at Birmingham, Birmingham, AL.

[7]Division of Infectious Diseases, Department of Medicine, Brown University, Providence, RI.

Dr. Carcillo received support for article research from the National Institutes of Health (NIH). His institution received grant support (support from National Institute of General Medical Sciences (NIGMS) for his research related to multiorgan dysfunction syndrome [MODS] and support from NIGMS for his research in MODS/macrophage activation syndrome [MAS]). Dr. Chatham's institution received grant support from Swedish Orphan Biovitrum (SOBI) (pending grant for clinical trial re: anakinra in MAS). Dr. Cron provided expert testimony for Wicker Smith O'Hara McCoy and Ford P.A. (peds rheum case expert evaluation). His institution received grant support from SOBI (clinical trial of anakinra for MAS). Dr. Dinarello served as a board member for BioTechne. Dr. Opal consulted for BioAegis (advice on phase 2 study in severe pneumonia), Battelle (advice on preclinical development on hemoperfusion columns for sepsis), Biocardis (advice on rapid blood steam genomic diagnostic methodologies), ImmuneXpress (advice on rapid diagnosis of sepsis based on host response gene response), Grifolds (advice on protein C use in sepsis studies), Octopharma (advice on plasma component therapy in sepsis), and Becton Dickinson (advice on new therapies for multidrug-resistant bacterial pathogens). He received royalties from Elsevier Publishers (royalties for a textbook in infectious diseases) and received support from Archeogen and Ketotek (data and safety monitoring boards). His institution received grant support from Asahi Kasei (clinical coordinating center for their phase 3 trial in sepsis) and Cardeas (clinical coordinating center for their phase 2 trial in severe pneumonia), Arsanis (preclinical study of monoclonal antibodies for blood stream infection). The remaining authors have disclosed that they do not have any potential conflicts of interest.

Address requests for reprints to: Bita Shakoory, MD, 2150 Pennsylvania Ave, NW. #G-404. Washington, DC 20037. E-mail: bshakoory@mfa.gwu.edu

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Objective: To determine the efficacy of anakinra (recombinant interleukin-1 receptor antagonist) in improving 28-day survival in sepsis patients with features of macrophage activation syndrome. Despite equivocal results in sepsis trials, anakinra is effective in treating macrophage activation syndrome, a similar entity with fever, disseminated intravascular coagulation, hepatobiliary dysfunction, cytopenias, and hyperferritinemia. Hence, sepsis patients with macrophage activation syndrome features may benefit from interleukin-1 receptor blockade.

Design: Reanalysis of deidentified data from the phase III randomized interleukin-1 receptor antagonist trial in severe sepsis.

Setting: Multicenter study recruiting through 91 centers from 11 countries in Europe and North America.

Patients: Sepsis patients with multiorgan dysfunction syndrome and/or shock (original study) were regrouped based on the presence or the absence of concurrent hepatobiliary dysfunction and disseminated intravascular coagulation as features of macrophage activation syndrome. The non–hepatobiliary dysfunction/disseminated intravascular coagulation group included patients with only hepatobiliary dysfunction, only disseminated intravascular coagulation, or neither.

Intervention: Treatment with anakinra or placebo.

Measurements and Main Results: Main outcome was 28–day mortality. Descriptive and comparative statistics were performed. Data were available for 763 adults from the original study cohort, randomized to receive either anakinra or placebo. Concurrent hepatobiliary dysfunction/disseminated intravascular coagulation was noted in 43 patients (5.6% of total; 18–75 years old; 47% women). The 28-day survival was similar in both anakinra and placebo-treated non–hepatobiliary dysfunction/disseminated intravascular coagulation patients (71.4% vs 70.8%; $p = 0.88$). Treatment with anakinra was associated with significant improvement in the 28-day survival rate in hepatobiliary dysfunction/disseminated intravascular coagulation patients (65.4% anakinra vs 35.3% placebo), with hazard ratio for death 0.28 (0.11–0.71; $p = 0.0071$) for the treatment group in Cox regression.

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Conclusions: In this subgroup analysis, interleukin-1 receptor blockade was associated with significant improvement in survival of patients with sepsis and concurrent hepatobiliary dysfunction/disseminated intravascular coagulation. A prospective randomized trial using features of macrophage activation syndrome for mortality risk stratification should be undertaken to confirm the role of interleukin-1 blockage. (*Crit Care Med* 2016; 44:275–281)

Key Words: anakinra; disseminated intravascular coagulation; hepatobiliary dysfunction; interleukin-1 receptor antagonist; macrophage activation syndrome; multiorgan dysfunction syndrome; sepsis mortality

"Sepsis" constitutes various degrees of systemic inflammation in the context of microbial infection (1), with production of proinflammatory cytokines including interleukin-1 (IL-1), IL-6, IL-18, and tumor necrosis factor (2, 3). These cytokines may contribute to development of sepsis-induced multiorgan dysfunction syndrome (MODS) and/or hypoperfusion, identified as severe sepsis and septic shock, respectively, based on International Sepsis Definitions Conference guidelines (1).

Following the discovery of IL-1 as human leukocytic pyrogen in 1977 (4), IL-1 receptor antagonist (IL-1Ra) was purified in 1981 (5) and was demonstrated to have an important role in regulating systemic inflammatory responses (3–6). IL-1Ra (3, 7) is a 25 kD naturally occurring plasma protein that binds to the type 1 IL-1 receptor, inhibiting both IL-1α and IL-1β signal transduction (7). Anakinra is a 17-kD recombinant, nonglycosylated human IL-1Ra. Prior to its approval to treat other inflammatory diseases (8), anakinra was investigated in a series of clinical trials for treatment of patients with sepsis and severe sepsis (9–12). An initial phase I study was conducted with IV doses as high as 10 mg/kg/hr for 72 hours (9), which established the safety and tolerability of anakinra at high doses. A subsequent phase II open-label clinical trial demonstrated dose-related efficacy in sepsis syndrome and septic shock without treatment-related adverse outcomes (10). In an ensuing phase III randomized double-blind trial comparing 1 or 2 mg/kg/hr with placebo, anakinra failed to meet the primary endpoint of improved 28-day survival (11), but did improve survival in patients with MODS, a predicted mortality probability of greater than 24% at study entry, or septic shock (11). On the basis of these results, a subsequent confirmatory phase III trial was undertaken in patients with severe sepsis or septic shock (12). This latter study was discontinued following an interim analysis, in which no survival benefits could be demonstrated (12). This, and the other clinical trials of anakinra in sepsis, did not reveal either increased mortality rate or serious/nonserious adverse reactions in anakinra over placebo groups (9–12).

In contrast to its effects in sepsis, anakinra, either alone or in combination with other treatments, has been reported to be effective in the management of macrophage activation syndrome (MAS), previously termed "secondary or reactive hemophagocytic lymphohistiocytosis" (13). Clinically, MAS presents as a fulminant cytokine storm and often fatal cause of MODS (13–17), associated with pancytopenia, tissue hemophagocytosis, liver dysfunction, coagulopathy, and/or CNS dysfunction (18–22), occurring as a complication of infection, malignancies, and autoimmune disorders (13, 15). The excessive inflammatory response in MAS is reflected in the amplified transcription of ferritin, and tissue-bound and soluble CD163 (23–25), both of which have also been demonstrated in severe sepsis (26–31).

Given the clinical and cytokine similarities of MAS and severe sepsis, together with the observed favorable response of MAS to anakinra, the possibility that severe sepsis patients with features of MAS may respond to IL-1R blockade merits consideration. This study revisits the data from the confirmatory phase III randomized clinical trial of IL-1Ra (anakinra) in severe sepsis (12), reanalyzing the data to investigate the response to anakinra in patients with features of MAS (hepatobiliary dysfunction [HBD] and disseminated intravascular coagulation [DIC]) (18–22).

PATIENTS AND METHODS

Data Source

The data from the previously published original confirmatory phase III placebo-controlled trial of IL-1Ra in severe sepsis (12) were reanalyzed. In this trial, 906 patients with severe sepsis and septic shock were recruited from 91 centers in 11 countries across Europe and North America, randomly assigned to either treatment or placebo (1:1 ratio). The primary endpoint was defined as 28-day survival; end-organ dysfunction and/or failure were determined by consensus of a steering committee according to the guidelines in the antecedent phase III clinical trial (11) with additional modifications (12). The original study was stopped at the second interim analysis for futility. At the time of study termination, 58 patients had completed the 3-day infusion (drug or placebo) and were in various stages of 28-day follow-up. A group of 143 patients was in various stages of screening and infusion, without completing the full infusion course. This group was dropped from the study, and their data were not available for the current analysis.

Regulatory Process

Each participating center obtained institutional review board approval, and all subjects gave written informed consent to participate in the original trial. The dataset included demographic, clinical, and outcome data for subjects in the study, each identified by a study-specific number. None of the personal identifiers were disclosed in the dataset that was used for this study.

Variables

The available data for each subject used for this study included age, gender, presence or absence of shock, DIC, HBD, or acute kidney injury (AKI), survival at day 28, predicted mortality, and treatment. Definitions of DIC and organ dysfunction (HBD, shock, acute respiratory distress syndrome [ARDS], and AKI) were as described previously in the original open-label trial (11) (**Table 1**). The definitions of shock and DIC were amended in the confirmatory phase III clinical trial (12). Twenty-eight–day

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survival was the primary endpoint of this study. The Acute Physiology and Chronic Health Evaluation (APACHE) II score was used to calculate predicted death, which strongly correlated with APACHE II (11, 12). The APACHE II score and the specifics of infecting organisms were not available as part of the dataset. All of the subjects with missing data for treatment, 28-day outcome, or both DIC and HBD domains were excluded, as these were the main variables of interest in this study.

Disease and Nondisease Groups

In keeping with the objective of the study, features of MAS were defined as the presence of both HBD and DIC in this severe sepsis cohort. This choice of definition was guided by the clinical characteristics of MAS as determined in major studies (18–22), and each patient was classified into one of the two main groups of interest:

- HBD/DIC group: patients whose clinical presentation met the definitions of both HBD and DIC and
- non-HBD/DIC group: patients who did not have both HBD and DIC and included patients with only HBD, only DIC, or neither.

Treatment Versus Placebo

The original study was a randomized, placebo-controlled study. Patients in each group had, therefore, been randomly assigned at a 1:1 ratio to receive either placebo or anakinra, administered IV at 2.0 mg/kg/hr for 72 hours continuously.

Statistical Analysis

Statistical analysis was performed to compare demographic, clinical, and outcome variables between four subgroups comprised HBD/DIC and non-HBD/DIC patients who received anakinra or placebo. Descriptive statistics was performed for the variables, using mean and SD for continuous variables (age and predicted death), or proportion and percent for binomial variables. Comparative statistics included *t* tests for continuous variables, as well as Fisher Exact test and chi -square test for binomial variables. Kaplan-Meier survival estimates were also compared between the drug and control groups who were HBD/DIC positive, HBD/DIC negative, or HBD/DIC equivocal. The possibility of postrandomization imbalances was investigated by comparing pretreatment variables in drug versus control groups, using between-group *t* test or chi-square test. Drug effects were

TABLE 1. Variable Definitions Used in the Original Study by Opal et al (12)

Variable	Definition
Shock	Either of the following: • Systolic blood pressure ≤ 90 mm Hg, mean arterial pressure ≤ 70 mm Hg, decrease in systolic blood pressure ≥ 40 mm Hg • Persistent need for vasopressor agents to maintain blood pressure (excluding dopamine at dosages < 5.0 μg/kg/min) despite adequate fluid resuscitation
Disseminated intravascular coagulation	Both criteria No anticoagulation • PT or partial thromboplastin time ≥ 1.2 × normal value • Platelet count ≤ 100×10^9/L or ≥ 50% decrease in platelet count With therapeutic doses of warfarin or heparin • Abnormal platelet counts (above) and • Elevated fibrin split products or the D-dimer ≥ 0.5 mg/L
Acute kidney injury	Any of the following • Serum creatinine concentration ≥ 177 mmol/L (≥ 2.0 mg/dL) • Need for dialysis • Pre-existing renal dysfunction: ≥ 177 mmol/L (≥ 2.0 mg/dL) increase in the serum creatinine concentration
Hepatobiliary dysfunction	At least two of the following • Total serum bilirubin concentration ≥ 43 mmol/L (≥ 2.5 mg/dL) • Serum concentration of aspartate aminotransferase or alanine aminotransferase ≥ 2 × upper limit normal • PT ≥ 1.5 × normal value
Acute respiratory distress syndrome	According to criteria of Murray et al (47)

PT = prothrombin time.

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examined while controlling for potential confounders using logistic regression, as well as Cox proportional hazards models. A logistic regression model predicting 28-day survival was examined in the HBD/DIC subgroup to determine the association of drug with outcome, after controlling for potentially confounding covariates. To test whether the drug effect differed significantly across patients who were HBD/DIC positive, HBD/DIC negative, or HBD/DIC equivocal (defined as having either HBD positive or DIC positive but not both), we also tested a logistic regression model in the full sample using a drug × diagnosis interaction term in the model. To rule out the possibility that multicolinearity might have affected our multivariate models, we tested for this by examining variance inflation in weighted regression. SAS version 9.3 (Cary, NC) was used for all analyses. A nominal p value of less than 0.05 between groups was considered statistically significant.

RESULTS

Subject Group

Deidentified data were available on 763 study patients who had completed the study. All of these study patients were included in the analysis. The necessary variables for the current subgroup investigation were available in all of the patients. Of these, 43 patients (46.6% women) demonstrated both HBD and DIC; 26 (60.4%) were treated with anakinra (17 placebo). The remaining 720 subjects (40.7% women) were similarly distributed in anakinra or placebo groups: 484 subjects (67.2%) on anakinra, 236 subjects receiving placebo (**Table 2**). Despite the planned 1:1 randomization of the patients in the original trial, the observed ratio of anakinra to placebo group in the studied patient cohort was 2:1. A similar distribution is observed in the HBD/DIC subgroup. This has been confirmed with the investigators of the study to be merely a chance finding, likely related to early termination of the study.

Patient Characteristics in HBD/DIC and Non-HBD/DIC Groups

Clinical and outcomes characteristics of the patients are reported in the original study publication (12). The descriptive details of the subgroups reported herein are outlined in Table 2. In comparative analysis, the HBD/DIC group subjects were significantly younger than their non-HBD/DIC counterparts ($p = 0.025$) with significantly higher predicted mortality scores at study entry ($p = 0.0001$). The HBD/DIC patients were less likely to be alive at 28 days (53.5% vs 70.0%; $p = 0.027$). Average survival duration in HBD/DIC patients was 16.5 days, significantly shorter than the 21.7 days in non-HBD/DIC patients ($p = 0.0004$). ARDS was noted in similar proportions in both groups, but AKI and shock were both more frequent among the HBD/DIC patients ($p = 0.0001$ for AKI; $p = 0.0089$ for shock).

Mortality and Treatment With Anakinra

The outcome of death related to anakinra versus placebo treatment was determined over the course of the study observation period for HBD/DIC and non-HBD/DIC patients. As shown in **Figure 1**, 28-day mortality was equivalent for the non-HBD/DIC patients who received anakinra (29.7%) or placebo (30.5%; $p = 0.999$). However, the 28-day mortality in HBD/DIC patients who received anakinra was significantly lower (34.6%) than was noted for HBD/DIC patients who received placebo (64.7%; $p = 0.0006$), corresponding to a 47% reduction in mortality associated with anakinra.

Confirmatory Analysis

Given the post hoc nature of this investigation, confirmatory statistical evaluations were performed to identify any postrandomization imbalances or confounders. We confirmed that the predicted death probability score was normally distributed with a mean 0.36 ± 0.21 and a median = 0.32, in the full sample. Probability of death was well balanced between treatment and placebo groups (mean predicted death: 0.358 ± 0.21 in the treated group [$n = 510$] and 0.354 ± 0.21 in the placebo group [$n = 253$]; $p = 0.81$). The risk of death between HBD/DIC patients who received anakinra treatment (mean, 0.57 ± 0.22) versus placebo group (0.53 ± 0.25) was also similar in t test comparison ($p = 0.54$). Other covariate values are shown for HBD/DIC-positive patients stratified by treatment in **Table 3**. None of these were statistically significant. Taken together, these findings indicate the treatment groups are balanced on risk of death and other potential confounding variables.

To further confirm the results, the patients were divided into three groups including HBD/DIC positive ($n = 43$), HBD/DIC negative ($n = 470$), who have neither HBD nor DIC, and 217 who have either HBD or DIC positive but not both (HBD/DIC equivocal). There is a large significant difference in 28-day survival between these three groups, with HBD/DIC-positive patients having the lowest survival rate (54%), followed by HBD/DIC-equivocal patients (58%), and HBD/DIC-negative patients having the highest survival rate (77%; chi-square = 32.1; $p < 0.0001$; phi = 0.21).

In univariate analysis (using chi-square for categorical variables and t test for age), age ($p < 0.0001$), AKI ($p < 0.0001$), and ARDS ($p = 0.0066$) are associated with 28-day mortality, whereas gender ($p = 0.73$), infectious cause ($p = 0.66$), shock ($p = 0.22$), and sepsis cause ($p = 0.21$) are not. Therefore, age, AKI, and ARDS were used as covariates in examining the association of drug with 28-day mortality in HBD/DIC patients.

In the logistic regression model in HBD/DIC-positive patients, the drug was associated with a reduced rate of death in HBD/DIC patients, after controlling for covariates, with an adjusted odds of 28-day mortality 87% lower than HBD/DIC patients on placebo (odds ratio for death, 0.13 [0.03–0.71]; $p = 0.018$).

Also in the logistic regression model using all patients, the diagnosis × treatment interaction was significant ($p = 0.025$), indicating different drug effects on mortality, depending on HBD/DIC status. Treatment with anakinra was associated with increased incidence of 28-day mortality in HBD/DIC-equivocal patients when compared with that in controls, but anakinra therapy was associated with reduced incidence of 28-mortality in HBD/DIC-positive patients when compared with that in non-HBD/DIC controls. The

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TABLE 2. Characteristics and Associations of Patients Dichotomized Based on Presence or Absence of Hepatobiliary Dysfunction/Disseminated Intravascular Coagulation

	All ($n = 763$)	HBD/DIC ($n = 43$)	Non-HBD/DIC ($n = 720$)	p
Mean age (yr) (range)	57.9 (17–95)	52.3 (18–75)	58.3 (17–95)	**0.025**
Female sex (%)	313 (41)	20 (46.5)	293 (40.7)	0.523
Anakinra (recombinant interleukin-1 receptor antagonist) treatment (%)	510 (66.8)	26 (60.4)	484 (67.2)	NA
Mean survival day	21.4	16.5	21.7	**0.0004**
Predicated death (range)	0.346 (0.015–0.986)	0.553 (0.143–0.986)	0.345 (0.015–0.95)	**0.0001**
Alive in day 28	527 (69.1)	23 (53.5)	504 (70.0)	**0.0273**
Concurrent dysfunctions (%)				
Acute respiratory distress syndrome	194 (25.4)	9 (20.9)	185 (25.7)	0.590
Acute kidney injury	232 (30.4)	26 (60.5)	206 (28.6)	**0.0001**
Shock	613 (80.3)	41 (95.3)	572 (79.4)	**0.0089**

HBD = hepatobiliary dysfunction, DIC = disseminated intravascular coagulation.
Statistically significant $p = 0.05$. Boldface font indicates statistically significant values.

multicolinearity test found all variance inflation factor less than 1.2 for all predictors, indicating no issue with multicollinearity.

In the Cox regression analysis of HBD/DIC-positive patients only, treatment was significantly associated with time to death ($p = 0.0071$) with a hazard ratio of 0.28 (0.11–0.71) for anakinra versus placebo groups, after adjusting for age, AKI, ARDS, and death risk score. In Cox regression in the full sample, using six groups of HBD/DIC × treatment (HBD/DIC positive, HBD/DIC negative, or HBD/DIC equivocal by treatment [anakinra vs placebo]) with HBD/DIC-positive placebo treated as the reference group, the hazard ratio for the HBD/DIC-positive treatment group was 0.28 (0.12–0.68; $p = 0.0051$) after adjusting for age, AKI, ARDS, and risk score. This indicates that the HBD/DIC-positive patients who received the drug had a significantly reduced likelihood of 28-day mortality when compared with HBD/DIC patients in the placebo group. Kaplan-Meier survival estimates for the six combinations of HBD/DIC and anakinra demonstrate the survival advantage for the HBD-positive/anakinra group over the HBD-positive/placebo group begins on day 1 and becomes more apparent on day 5.

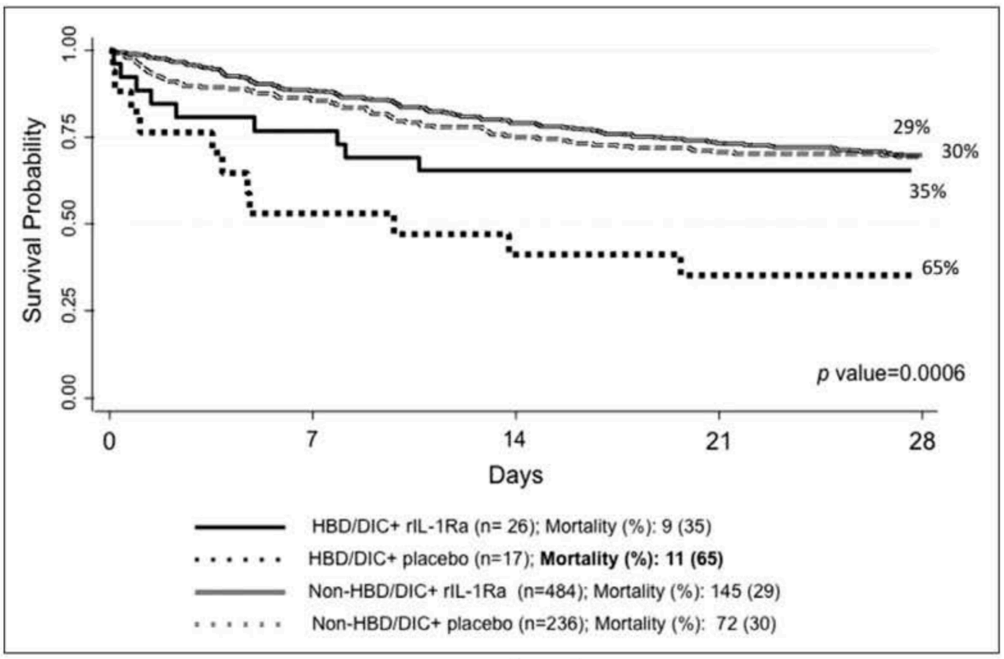

Figure 1. Treatment with interleukin-1 receptor antagonist (IL-1Ra) significantly improves 28-d survival in hepatobiliary dysfunction (HBD)/disseminated intravascular coagulation (DIC) sepsis, but does not change the outcome in non–HBD/DIC sepsis. rIL-1Ra = anakinra (recombinant interleukin-1 receptor antagonist), HBD/DIC group = patients with severe sepsis who demonstrate both HBD and DIC features, non–HBD/DIC group = patients with either HBD alone (no DIC), DIC alone (no HBD, or neither). Statistically significant $p = 0.05$.

DISCUSSION

The benefits of IL-1R blockade in modifying the detrimental effects of the inflammatory process have been demonstrated since 1984 (3); yet, the appropriate target population among sepsis/MODS patients who would benefit from treatment with anakinra has remained elusive. The recent recognition of and

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TABLE 3. Values of Pretreatment Variables in Treatment Versus Control Groups in Patients With Hepatobiliary Dysfunction/Disseminated Intravascular Coagulation

Variable	Treatment ($n = 26$)	Control ($n = 17$)	p
Age (yr)	49.6 ± 12.7	56.3 ± 19.4	0.18
Female sex (%)	12 (46.2)	8 (47.1)	0.95
Acute kidney injury (%)	17 (65.4)	9 (52.9)	0.41
Acute respiratory distress syndrome (%)	6 (23.1)	3 (17.7)	0.28
Risk of death	0.57 ± 0.22	0.53 ± 0.25	0.54

development of treatments for MAS may be informative because MAS and severe sepsis share many clinical, laboratory, and pathologic features, including elevated serum ferritin (28, 29), cytopenias, hepatic dysfunction, coagulopathy (28, 29), CNS dysfunction (30), tissue hemophagocytosis (31), and elevated macrophage expression of CD163+ cells throughout the reticuloendothelial system (27, 32). These shared clinical/laboratory features of MAS and severe sepsis occur in the context of a cytokine storm in which IL-1 plays a major role (2, 3, 6, 15).

Anakinra is a recombinant human IL-1Ra with a short half-life of about 3–4 hours (7), wide therapeutic range (7), and high safety profile (7). The use of anakinra as a treatment option for MAS was initially reported in 2006 (33) with subsequent case reports/series reporting the benefits of IL-1R blockade, mainly in children with MAS complicating systemic juvenile idiopathic arthritis and adults with Still disease (34–44). It is, therefore, plausible that a subset of severe sepsis patients develop MAS, and that the associated severe organ dysfunction is responsive to IL-1 blockade with anakinra.

In recent studies to develop validated case definitions for MAS, attributes of HBD (liver enzyme elevation) and DIC (hypofibrinogenemia and thrombocytopenia) are consistently among the most prevalent clinical features encountered in patients with presumed MAS (18, 20). Both HBD and DIC were readily assessed in the original anakinra/sepsis phase III study data, permitting the use of the presence of HBD and DIC as a marker for possible MAS in the current analysis.

Mortality rates of adult MAS have been reported as up to 60% in various studies (21, 22) comparable with the observed mortality rate noted in the HBD/DIC patients randomized to placebo. Treatment with anakinra reduced mortality in HBD/DIC patients when compared with the mortality rate of non-HBD/DIC patients, but did not lead to a meaningful difference in survival in non-HBD/DIC patients when compared with those randomized to placebo. These findings suggest severe sepsis patients with HBD/DIC may represent a state of cytokine storm similar to MAS and high mortality risk, who may be the most appropriate patients to receive immune modulating and cytokine-targeted therapies. The identification of the appropriate subgroup of sepsis patients to target for potentially life-saving anticytokine targeted therapy provides a rationale for future prospective investigations to verify the use of IL-1 blockade in sepsis associated with features of MAS.

When compared with activated protein C (45) as an intervention for sepsis, anakinra has no significant reported safety issues since introduction in the market in 2001 (46), with higher survival benefits in the selected population in this study and a lower number needed to treat. Although the subset analysis undertaken herein offers suboptimal power, these favorable findings merit future randomized studies to establish the benefits of anakinra in sepsis patients with features of MAS and MAS patients who have complicating sepsis.

CONCLUSIONS

The present findings support the possibility that anakinra treatment provides a survival benefit in septic patients with features of MAS. The safety and wide therapeutic margin of anakinra and the central role of IL-1 in the cytokine storm in severe sepsis patients merit reconsideration of this therapeutic as a potential treatment option in carefully selected groups of sepsis patients with attributes of MAS.

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MAJOR ARTICLE

Ebola Hemorrhagic Fever: Novel Biomarker Correlates of Clinical Outcome

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Background. Ebola hemorrhagic fever (EHF) outbreaks occur sporadically in Africa and result in high rates of death. The 2000–2001 outbreak of Sudan virus–associated EHF in the Gulu district of Uganda led to 425 cases, of which 216 were laboratory confirmed, making it the largest EHF outbreak on record. Serum specimens from this outbreak had been preserved in liquid nitrogen from the time of collection and were available for analysis.

Methods. Available samples were tested using a series of multiplex assays to measure the concentrations of 55 biomarkers. The data were analyzed to identify statistically significant associations between the tested biomarkers and hemorrhagic manifestations, viremia, and/or death.

Results. Death, hemorrhage, and viremia were independently associated with elevated levels of several chemokines and cytokines. Death and hemorrhage were associated with elevated thrombomodulin and ferritin levels. Hemorrhage was also associated with elevated levels of soluble intracellular adhesion molecule. Viremia was independently associated with elevated levels of tissue factor and tissue plasminogen activator. Finally, samples from nonfatal cases had higher levels of sCD40L.

Conclusions. These novel associations provide a better understanding of EHF pathophysiology and a starting point for researching new potential targets for therapeutic interventions.

Keywords. Ebola virus; biomarkers; hemorrhage; Gulu; hemorrhagic fever virus.

Ebola hemorrhagic fever (EHF) was first identified in 1976 during an outbreak in the Democratic Republic of the Congo (formerly known as Zaire). Since then, 21 additional outbreaks among humans have been recorded in sub-Saharan Africa. The largest of these outbreaks took place in the Gulu district of Uganda in 2000–2001 and was caused by Sudan virus (SUDV). This outbreak resulted in 425 cases, 216 of which were laboratory confirmed, and a 53% overall case-fatality rate [1].

The genus *Ebolavirus* includes 5 different viruses: SUDV, Tai Forest virus (TAFV), Reston virus (RESTV), Ebola virus (EBOV), and Bundibugyo virus (BDBV). EHF caused by EBOV has the highest fatality (57%–90%), followed by SUDV (41%–65%) and BDBV (40%); to date, RESTV infection has been asymptomatic in humans, and TAFV has only been identified in 2 human infections, both of which were nonfatal [2–4]. Clinical EHF is characterized by abrupt onset of fever, fatigue, headache, myalgia, and gastrointestinal distress 3–13 days after exposure to virus [5]. Many patients develop hemorrhagic manifestations, which has led to the term "hemorrhagic fever."

The samples collected during the Gulu outbreak have already yielded extensive information about EHF pathophysiology, including the observation that aspartate aminotransferase (AST), D-dimer, blood urea nitrogen (BUN), and creatinine levels are higher than normal, while calcium and albumin levels are lower than normal in samples from fatal EHF cases [6]. Elevated levels of the cytokines interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 10 (IL-10), and macrophage inflammatory protein 1β (MIP-1β) were also associated with fatal outcomes [7]. Additionally, human leukocyte antigen B67 (HLA-B67), HLA-B15, and marked CD8

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lymphopenia were associated with fatal outcome, and HLA-B7 and HLA-B14 were associated with nonfatal outcome [8, 9].

The goal of our study was to better understand the pathophysiology of EHF by correlating hemorrhagic manifestations and death with specific biomarkers. We measured levels of markers of immune function, endothelial activation, and coagulation in available patient serum samples, to determine whether alterations in levels of these biomarkers were associated with hemorrhagic manifestations, viremia, or death.

METHODS

Study Design

During the 2000–2001 Gulu outbreak, an international response team, including Centers for Disease Control and Prevention (CDC) staff, responded with clinical and technical assistance. Serum samples were frozen in liquid nitrogen in the field and have been stored that way since the time of the outbreak. For our analyses, samples were chosen to represent sex ratios, hemorrhagic manifestations, and death rates consistent with those observed during the outbreak (Table 1). Specimens were prioritized for novel analyses; serum chemistry analyses were performed on samples of sufficient volume. All samples were inactivated by γ irradiation before use.

Institutional Review Board (IRB)

IRB approval was obtained before initiating the study, and an exemption was granted by the CDC Human Research Protection Office.

Luminex-Based Assays

The following assays were purchased from Affymetrix (Santa Clara, CA) and performed according to the manufacturer's instructions: a 26-plex assay for granulocyte macrophage colony stimulating factor (GM-CSF), GROα, interferon α2 (IFN-α2), IFN-β, IFN-γ, IL-10, interleukin 12p70 (IL-12p70), IL-12p40, interleukin 1α (IL-1α), IL-1β, IL-1 receptor antagonist (IL-1RA), interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 5 (IL-5), IL-6, IL-8, IFN-γ–inducible protein 10 (IP-10), monocyte chemoattractant protein 1 (MCP-1), macrophage colony-stimulating factor (MCSF), MIP-1α, MIP-1β, soluble CD40 ligand (sCD40L), soluble E-selectin (sE-selectin), soluble Fas ligand (sFasL), tumor necrosis factor α (TNF-α), and vascular endothelial growth factor A (VEGF-A); a 2-plex assay for D-dimer and tissue plasminogen activator (TPA); a 5-plex assay for plasminogen activator inhibitor-1 (PAI-1), serum amyloid antigen (SAA), regulated on activation, normal T-cell expressed and secreted (RANTES), soluble intracellular adhesion molecule 1 (sICAM-1), and soluble vascular cell adhesion molecule (sVCAM-1); and single-plex assays for C reactive protein (CRP) and fibrinogen. Assays for ferritin and cortisol were performed as single-plex assays according to manufacturer's instructions (Millipore, Billerica, MA). 2-plex assays were performed for tissue factor (TF) and thrombomodulin according to the manufacturer's instructions (Millipore). For samples with values outside the upper end of the standard curve, additional dilutions were made as necessary to obtain accurate values for all analytes.

Table 1. Patient Characteristics

Characteristic	This Study (n = 86)	Gulu Outbreak (n = 425)
Age, y, range	6–60	0.25–81
Sex		
Female	56 (65)	267 (63)
Male	30 (35)	158 (37)
Any hemorrhagic manifestation	31 (36)	128 (30)
Death	41 (48)	224 (53)

Data are no. (%) of patients, unless otherwise indicated. Gulu outbreak data are from Okware et al [1].

Enzyme-Linked Immunosorbent Assays (ELISAs)

Mannose-binding lectin (MBL; Hycult Biotech, Plymouth Meeting, PA) and total immunoglobulin G (IgG; eBioscience, San Diego, CA) ELISAs were performed according to the manufacturers' instructions. For samples with values outside the upper end of the standard curve, additional dilutions were made as necessary to obtain accurate values for all analytes.

Serum Chemistry Analyses

A Piccolo comprehensive metabolic reagent disc was run on the Piccolo xpress Chemistry Analyzer (Abaxis, Union City, CA) to determine serum chemistry values for alanine aminotransferase (ALT), albumin, AST, alkaline phosphatase, calcium, chloride, creatinine, glucose, potassium, sodium, total bilirubin, total CO_2, total protein, and BUN. Only 132 of the 187 samples had sufficient volume for serum chemistry analyses.

Human Immunodeficiency Virus (HIV) Testing

The Multispot HIV-1/HIV-2 Rapid Test (BioRad, Hercules, CA) was used to assess the HIV status of the patient cohort. Two patients (ages 7 and 19 years) did not have sufficient sample for analysis.

Viremia Analysis via Measurement of RNA Copy Number

Total RNA was isolated from serum using the MagMAX-96 Viral RNA Isolation Kit on the MagMAX Express-96 Magnetic Particle Processor (Ambion, Grand Island, NY). In parallel, serial dilutions of a normal human serum specimen inoculated with a known titer of SUDV was subjected to RNA isolation and used to generate a standard curve. Real-time polymerase chain reaction was then performed as previously described [10].

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Statistical Analysis

Data on sex, age, date of symptom onset, viremia levels, hemorrhagic manifestations, HIV status, patient survivability, and date of sample collection were available for the 86 patients included in our study. Only samples taken 0–15 days from symptom onset were analyzed, to represent the normal course of the illness.

Analysis of variance (ANOVA) was conducted for each of the 55 analytes, using sex, age, day from symptom onset, viremia, death, hemorrhaging, and HIV status as the independent variables. All variables were converted to categorical variables (meaning that there was limited number of outcomes [ie, viremic vs not viremic]) because of the number of samples and the distribution of the numerical data, with the date from symptom onset converted to 3 categories (0–5 days, 6–10 days, and 11–15 days). An initial overall α of 0.25 was set, and the Bonferroni inequality was used to arrive at a critical P value of .0045 for the individual analytes. Using these criteria, 29 of the 55 analytes had statistically significant differences. Knowing the impact of multiple testing on the power of tests, the technique of Yoav and Hochbergs was used to control the false discovery rate to balance the power and type 1 error of the analyses [11]; this correction resulted in 10 additional analytes meeting the criteria for statistical significance. The independent variables were analyzed to measure colinearity, but no significant variance inflation was observed.

Ultimately, our study included 161 samples, each described by 9 categorical variables. Each sample was analyzed for 41 different analytes (one sample was insufficient to perform a subsequent analysis of 2 analytes). An analysis of metabolic information was attempted for all samples, but only 132 samples had sufficient volume for analyzing 14 additional analytes.

Eight analytes (IL-12p70, IL-12p40, IL-2, IL-4, IL-5, sFasL, IFN-γ, and IFN-α2) had statistically significant correlations but were excluded from analysis because $\geq$30% of the samples had a value below the limit of detection. We therefore did not attempt to make conclusions based on data that could have been affected by degradation over time.

Model Selection

After the initial ANOVA, 27 significant analytes were selected for further analysis. Stepwise regression for each significant analyte was used to find the best model, excluding insignificant variables at an α of 0.05. Particular attention was paid to those analytes in which age, death, viremia, hemorrhaging, and HIV status were significant. Ten analytes were significant for age, 16 for death, 7 for viremia, 8 for hemorrhaging, and 2 for HIV status. We compiled the analytes that were significantly different for these variables and calculated the means and standard error for each interval.

Finally, 2 additional statistical tests were performed to measure the relationships between death and both viremia levels and the presence of hemorrhaging. The distribution of the viremia levels was clearly nonnormal, so the Mann–Whitney U test was used to determine differences in viremia for patients with fatal and those with nonfatal outcomes. Patients with no measurable viremia were excluded from analysis. The survivability of patients with and those without hemorrhaging was also examined, but no significant differences were found.

RESULTS

Study Population

The study included 86 EHF patients from the 2000–2001 Gulu outbreak that were representative of the outbreak as a whole. As noted in previous outbreaks, more women were affected than men, likely because women are commonly caretakers, a known risk factor for contracting EHF. Thirty-six percent of the patients in this study had hemorrhagic manifestations (defined as any of the following: vomiting blood, blood in the stool, or bleeding from the gums, skin, or eyes), and approximately half of the patients died, consistent with reported death rates for SUDV (Table 1). One limitation to our study was that the limited sample volume influenced our selection of samples because we only selected patients for whom there were sufficient sample volume to complete the desired testing. This may have biased our data slightly toward the nonfatal group.

In the filovirus field, it has generally been assumed that immune system deficiencies associated with HIV infection would likely negatively impact EHF outcome. As the HIV status of EHF patients has never been determined previously, HIV testing was conducted on patients included in this study. None of the patients <18 years of age were HIV positive. Of the 67 adults (ages 18–60 years), 12 (17.9%) were HIV positive. This rate is roughly consistent with data on HIV prevalence in the Gulu region of Uganda during the time of the outbreak; 12% of women attending antenatal clinics were reported to be HIV positive [12]. Of the 12 HIV-positive adults, 5 were male and 7 were female. Although $CD4^+$ T-cell counts were unknown in these patients, 50% of the HIV-positive adults (3 males and 3 females) had fatal outcomes. This mortality rate was approximately the same as that in the total adult population, suggesting that an individual's HIV status did not significantly affect outcome during SUDV infection.

Study Design

Fifty-five different biomarkers were assessed in 187 serum samples in this study. Thirty-six patients had $\geq$2 serial samples collected 1–15 days after onset of symptoms; composite data rather than individual patient data are presented, to provide the most objective data for analysis. Supplementary Table 1 provides the statistical significance for each biomarker tested and its relationship to the examined variables. The data presented in this article represent the statistically significant associations observed

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Table 2. Coefficient of Variance (CV) Between Repeat Chemistry Measurements After 12 Years of Storage in Liquid Nitrogen

CV	ALT	AST	BUN	CRE	TP	ALB
Percentage difference, mean (range)	2.3 (0–7.4)	1.5 (0–4.4)	1.7 (0–7.1)	2.2 (0–5.4)	0.96 (0–2.9)	0.75 (0–2.8)

Abbreviations: ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CRE, creatinine; TP, total protein.

between a given biomarker and death, hemorrhagic manifestations, or viremia.

Serum Chemistry Analysis and Viremia

Serum chemistry analyses were performed on many patients as part of their clinical management during the 2000–2001 outbreak. These data were still available, allowing for a direct assessment of the stability of several metabolic analytes after 12 years of storage in liquid nitrogen. Many analytes were strikingly stable, as shown by a maximal coefficient of variance of 7.4% between the original measurement and the repeat measurement for ALT, AST, BUN, creatinine, total protein, and albumin levels (Table 2). The more labile components, CO_2 and electrolytes, were excluded from our analysis.

Previously reported associations between fatal outcomes and AST, BUN, creatinine, and albumin levels [6] were again seen in our study (data not shown) [6]. Higher levels of viremia were associated with a fatal outcome, as previously demonstrated [10] (Figure 1).

Cytokines and Chemokines

Cytokines and chemokines are a diverse, extensively studied group of proteins that modulate the immune response. We analyzed 25 different cytokines and chemokines. IL-1α, IL-1RA, IL-6, MCP-1, MCSF, and MIP-1α levels were all elevated in patients with a fatal outcome (Figure 2*A*–*E* and 2*G*).

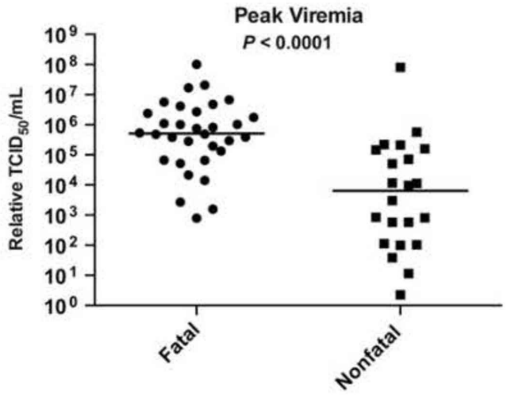

Figure 1. Viremia as a function of clinical outcome. Patients with fatal outcomes had higher viral loads. $TCID_{50}$, median tissue-culture infective dose.

Previously published data have shown associations between MIP-1β, IL-6, IL-8, and IL-10 levels and fatal outcomes in SUDV-infected patients [7]. All of these prior associations were observed in our study. However, IL-8 levels were only elevated very late in the infection in patients with a fatal outcome (data not shown); MIP-1β levels tended to be higher in patients with fatal outcomes but did not meet the cutoff for statistical significance; and IL-10 levels demonstrated an age-specific association with death. GRO-α levels, similar to IL-8 levels, were only elevated late during infection in patients who died (data not shown). IL-1α, MCSF, and IP-10 levels were elevated in samples with documented viremia, compared with samples with no detectable virus, but this finding was independent of their relationship with death (data not shown). MCSF, MIP-1α, and IP-10 levels were also elevated in patients who developed hemorrhagic manifestations (Figure 2*F*, 2*H*, and 2*I*). Unexpectedly, elevated levels of sCD40L correlated with nonfatal outcomes, a first for any report of EHF (Figure 2*J*).

Biomarkers of Inflammation

The acute phase response refers to a constellation of host responses that occur following infection and in other inflammatory diseases [13]. These responses are classically triggered by proinflammatory cytokines, such as IL-6, IL-1, IFN-γ, and TNF-α. Increased liver production of CRP and SAA and decreased production of albumin are common consequences of the acute-phase reaction. In addition, hypergammaglobulinemia and hyperferritinemia are often seen. These markers of inflammation are used clinically in many disease processes to help in diagnosis and to follow a patient's response to therapy. CRP levels were within the normal range in all samples and were excluded from further analysis. Ferritin levels were elevated in patients with fatal outcomes and in those with hemorrhagic manifestations (Figure 3*A* and 3*B*). Ferritin levels were also associated with viremia, independent of the associations with death and hemorrhage (data not shown). Ferritin levels were higher in HIV-positive patients than in HIV-negative patients (data not shown); this has been previously noted specifically in association with advanced HIV disease [14, 15]. HIV-positive patients also had higher total IgG levels than HIV-negative patients (data not shown), a well-documented phenomenon [16]. Finally, levels of sICAM, a marker of endothelial activation [17], were elevated in patients with hemorrhagic manifestations (Figure 3*C*)

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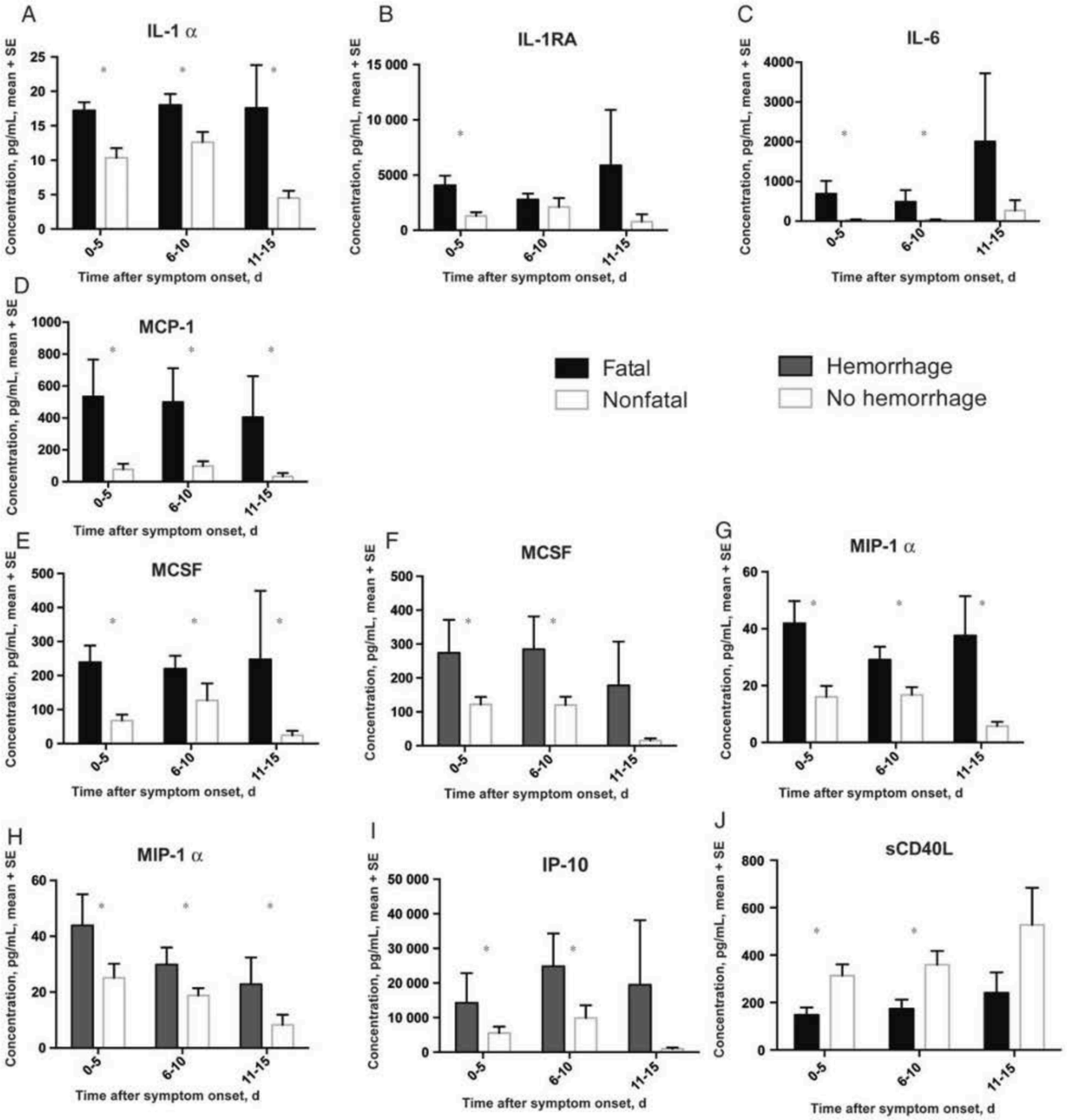

Figure 2. Cytokines and chemokines associated with a fatal outcome (black bars) or hemorrhagic manifestations (red bars). *$P \leq .05$. Abbreviations: IL-1α, interleukin 1α; IL-1RA, interleukin 1 receptor antagonist; IL-6, interleukin 6; IP-10, interferon γ–inducible protein 10; MCP-1, monocyte chemoattractant protein 1; MCSF, macrophage colony-stimulating factor; sCD40L, soluble CD40 ligand; SE, standard error.

Biomarkers of Coagulopathy

The frequency of hemorrhagic manifestations during EHF warranted an examination of the measureable factors that control hemostasis. The delicate balance of coagulation and fibrinolysis is depicted in a simplified model in Figure 4. In this study, PAI-1, fibrinogen, TPA, D-dimer, thrombomodulin, and TF levels were measured in all patient samples. TPA, D-dimer, thrombomodulin, and TF levels were all statistically significant in our analysis. Thrombomodulin levels were elevated in patients with fatal outcomes and in patients with hemorrhagic manifestations (Figure 4*A* and 4*B*). D-dimer levels were elevated in patients with a fatal outcome (Figure 4*C*), as previously reported [6]. TPA levels were higher in viremic patients (Figure 4*D*). TF levels were within the normal range in all samples

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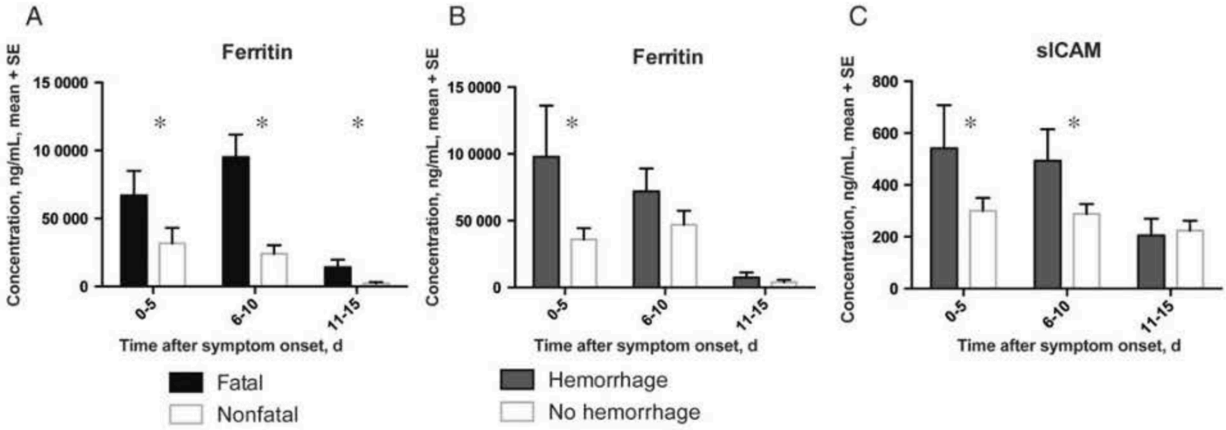

Figure 3. Markers of inflammation associated with a fatal outcome (black bars) or hemorrhagic manifestations (red bars). $^{*}P \leq .05$. Abbreviations: SE, standard error; sICAM, soluble intracellular adhesion molecule 1.

tested but were slightly higher in samples with detectable viremia than in those without detectable viremia (Figure 4*E*). Notably, TF levels were not associated with hemorrhagic manifestations or death.

DISCUSSION

We found associations between multiple proinflammatory cytokines and increased death rates, as previously shown in humans [7, 18–20] and EBOV-infected nonhuman primates [21], consistent with the idea of systemic inflammation contributing to a fatal outcome. We also found several novel associations, most strikingly, the association between increased sCD40L levels and nonfatal outcomes. Since sCD40L is largely produced by activated platelets [22], increased sCD40L levels could represent ongoing repair of altered endothelium by activated platelets. Additionally, nitric oxide (NO) inhibits platelet activation [23], and elevated NO levels have been reported in fatal EHF cases [9]. Therefore, the deleterious effects of NO could be related to inhibiting platelet activation, in addition to its vasodilatory effects. Effective repair of altered endothelium by activated platelets could lead to improved hemodynamic stability in patients and, thus, improved clinical outcomes.

Ferritin, another biomarker we examined, is a well-established acute-phase reactant that has been associated with severe disease in both dengue and Crimean Congo hemorrhagic fevers [24, 25]. Ferritin is the intracellular storage form of iron, and its levels increase during times of stress or inflammation. Ferritin levels were higher in samples from patients with EHF who had fatal outcomes, detectable viremia, and hemorrhagic manifestations; thus, ferritin could prove to be a useful clinical maker of EHF severity.

The striking clinical manifestation of hemorrhage during historic EHF outbreaks led to the naming of the disease as Ebola *hemorrhagic* fever, which has resulted in the perception that hemorrhage occurs in most patients and is associated with fatal outcomes. However, published data from the 3 largest EHF outbreaks, caused by EBOV (Kikwit, Democratic Republic of the Congo, 1995), SUDV (Gulu, Uganda, 2000), and BDBV (Bundibugyo, Uganda, 2007), demonstrate hemorrhagic manifestations in only 41%, 30%, and 46.5% of total cases, respectively [1, 3, 26]. Furthermore, no significant correlation between hemorrhage and death has been found in these same outbreaks of EBOV and BDBV infections. Consistently, we found no statistically significant differences in survival between patients with and those without hemorrhage ($P = .23$). Therefore, it is not surprising that, of the 6 different coagulation and fibrinolysis markers we assessed, only thrombomodulin was associated with both fatal outcome and hemorrhagic manifestations. Thrombomodulin is a marker of endothelial activation/dysfunction and, as discussed below, has many functions besides inhibiting coagulation. There is a common perception, not only in EHF but also in routine sepsis, that correction of coagulopathy will improve survival outcomes. However, therapeutic interventions that have focused on targeting coagulopathy in nonhuman primate models of EBOV only moderately increased survival [27, 28], and use of activated protein C in septic patients in the intensive care unit has been similarly disappointing [29]. A large focus has been placed on understanding the role of TF in EHF, given the marked increases in TF expression seen in monocytes in nonhuman primates infected with EBOV [30]. However, when TF levels were examined in patients from this outbreak, which was caused by SUDV, none of the patients had elevated levels of TF outside of the normal range, and there were no associations between TF levels and death or hemorrhage. These data are limited by our ability to only sample plasma TF versus monocyte-associated TF, although plasma and monocyte TF levels correlated in a human study of

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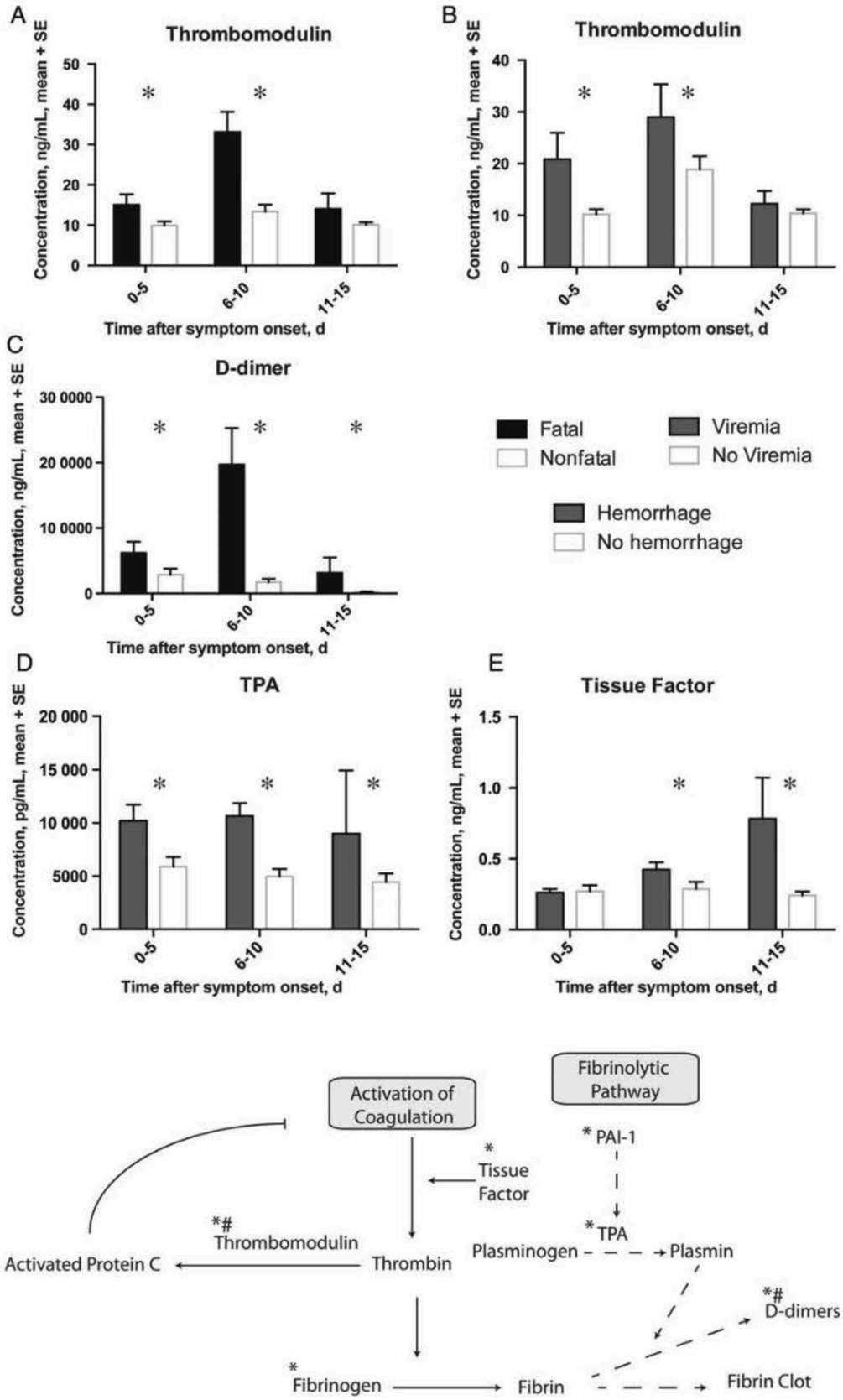

Figure 4. Markers of coagulation and fibrinolytic pathways associated with a fatal outcome (black bars), hemorrhagic manifestations (red bars) or viremia (blue bars). *$P \leq .05$. A simplified model of the coagulation/fibrinolytic pathway is also depicted. Proteins that are known to play a major role in the pathways are noted. Arrows indicate activation while bars indicate inhibition. *, analytes assessed in the study; #, analytes associated with death.

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thrombosis [31]. Finally, the nonhuman primate studies were performed with EBOV, whereas the patients in this study were infected with SUDV, so these TF differences could be virus or host related.

Thrombomodulin is released by an activated endothelium and functions as an anticoagulant by activating protein C when present in the microenvironment on the surface of endothelial cells. In our study, thrombomodulin levels were elevated in samples from patients who died and from those with hemorrhagic manifestations, the latter being consistent with its anticoagulant function. Thrombomodulin has several protein domains that are responsible for its anticoagulant activity, but it also contains a C-type lectin domain that has antiinflammatory activities, including maintaining endothelial integrity [32]. The fact that EBOV GP protein binds to other C-type lectins [33–35] makes the thrombomodulin C-lectin domain an attractive subject for further study. If thrombomodulin's C-lectin domain–mediated functions are inhibited by association with viral GP, this could provide a possible explanation for how these viruses are able to disrupt endothelial function without causing direct cytopathic effects. Alternatively, overactivation of endothelial cells, leading to shedding of thrombomodulin into the plasma, could result in a loss of thrombomodulin's stabilizing function at endothelial junctions, contributing to vascular leakage.

MCSF, MIP-1α, IP-10, sICAM, and thrombomodulin levels were all associated with hemorrhage in our study, and on the basis of findings in the literature, their activities can all be correlated with leukocyte/endothelium interactions. In one possible model, these cytokines could recruit leukocytes to areas of inflammation, and production of adhesion molecules such as ICAM would facilitate leukocyte adhesion, rolling, and diapedesis. Activated endothelial cells would then release thrombomodulin into the bloodstream, so it would no longer be present in the microenvironment to activate protein C and coagulation, nor would it be present to stabilize the endothelium. This would leave an activated, leukocyte-enriched, procoagulant endothelium, causing dysregulated hemostasis, which could manifest clinically as hemorrhage.

Future pathogenesis models could evaluate the possible role of activated platelets in improving clinical outcomes by therapeutic administration of platelets, inhibitors of NO synthetase, or active sCD40L to infected animals. Finally, the potential roles of thrombomodulin C-lectin domains in modulating EHF outcomes should be further studied at the molecular level to determine whether viral glycoproteins interact with thrombomodulin. If this proves to be the case, evaluating the role of thrombomodulin C-lectin domains in animal models would be warranted.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflict of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Does Viral Hemorrhagic Fever Represent Reactive Hemophagocytic Syndrome?

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Editorial

Does Viral Hemorrhagic Fever Represent Reactive Hemophagocytic Syndrome?

The recent Ebola virus disease (EVD) outbreak is the largest in our planet's history. The epidemic is centered in West Africa, but isolated cases are being recognized across continents and represent a global health crisis[1]. The mortality rate, once infected in non-hospitalized patients, is near 70%[2]. Over 11,000 individuals have died in the recent outbreak, and this likely represents an underreported figure. In addition to the ever-rising death toll, children in West Africa are being orphaned at an alarming rate. With no proven vaccine or curative therapy currently available and the possibility for spread to tens of thousands of individuals or more, there is a desperate and urgent need for novel therapy to combat this deadly disease, yet clinical trials are just getting under way.

Clinical Features of Viral Hemorrhagic Fever (VHF)

Ebola virus infection, following an incubation period of 2 to 21 days, is clinically characterized initially by fever, myalgia, headache, weakness, and abdominal pain. This is frequently followed by vomiting, diarrhea, and occasionally by bruising and bleeding (about 1 in 5 infected); thus the terminology, Ebola hemorrhagic fever.

The hemorrhagic complications seen in Ebola virus infection are also described in other specific virus families by the US Centers for Disease Control, including arenaviruses, filoviruses, bunyaviruses, and flaviviruses; hence, the term viral hemorrhagic fever is used to describe the severe multiorgan dysfunction syndrome (MODS) in a substantial number of infected individuals[3]. VHF shares striking similarities with hemophagocytic syndromes (HPS), both familial hemophagocytic lymphohistiocytosis (HLH) and reactive hemophagocytic syndrome (rHPS, also called macrophage activation syndrome, or MAS), which include the following: prolonged fever, coagulopathy, hyperferritinemia, liver enzyme elevations, increased serum interleukin 1 (IL-1) levels, thrombocytopenia, and central nervous system (CNS) dysfunction[4,5] (Table 1)[6]. Moreover, it has recently been elegantly shown in samples from the 2000–2001 Ebola outbreak of Sudan virus-associated VHF that hyperferritinemia, a hallmark of rHPS, was associated with hemorrhage and death[4].

Like Ebola virus, rHPS has been reported in association with other hemorrhagic fever viruses, such as Crimean-Congo and Dengue, with similar clinical (e.g., MODS including CNS dysfunction and coagulopathy)[5,7,8] and pathologic findings (e.g., hemophagocytosis)[9,10]. As in Ebola virus infection, hyperferritinemia has been observed to be a marker of hemorrhagic manifestation (and adverse outcome) in Dengue as well[11]. Collectively, features of VHF are seen in a substantial fraction of patients with certain viral illnesses such as Ebola virus, Dengue fever, Crimean-Congo fever, and other similar viruses. The clinical-pathological characteristics of the corresponding hemorrhagic fevers demonstrate striking resemblance with rHPS complicating viral illnesses. Despite the nonspecific nature of these findings, the constellation of these manifestations taken together is highly suggestive of rHPS. Thus, it can be speculated that VHF may in fact represent rHPS complicating the underlying viral illness.

Table 1. Similarities between hemophagocytic lymphohistiocytosis (HLH) 2004 criteria and features of viral hemorrhagic fever (VHF). Adapted from Chatham and Cron. The Rheumatologist 2014;8:1,21–23[6]; with permission.

HLH-04 Criteria	Features of VHF
Fever	Routinely
Splenomegaly	Sometimes
Bicytopenia or pancytopenia	Frequently
Hypertryglyceridemia or hypofibrinogenemia	Both have been reported for some hemorrhagic fever viruses
Hemophagocytosis on biopsy	Yes, for the viruses studied
Hyperferritinemia (> 500 ng/ml)	Yes, often > 10,000 ng/ml
Elevated soluble CD25	Yes, for the viruses studied
Absence of decreased natural killer cell function	Poorly studied to date

Pathophysiology of Hemophagocytic Syndromes

Our basis of understanding hemophagocytic syndromes, HLH and rHPS, is primarily derived from the study of familial HLH. Patients with familial HLH have homozygous or compound heterozygous defects in cytotoxic granule function through either defective granule contents, e.g. perforin, or defective granule transport and fusion machinery[12,13]. Work in genetic mouse models of familial HLH has led to a conceptual paradigm wherein defective cytotoxic killing by CD8 T cells and natural killer cells during infection leads to an inability to clear infection, and subsequent accumulation of infectious antigen[14,15]. This accumulation of antigen results in overstimulation of the frustrated lymphocytes, copious release of cytokines (a cytokine storm), and the resulting multiorgan pathology[13]. Such situations may arise when cytotoxic granule function is genetically intact as well[16]. Additionally, patients with systemic lupus erythematosus, systemic juvenile idiopathic arthritis, and

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other chronic inflammatory diseases may have more subtle abnormalities in cytotoxic immune cell function, perhaps exacerbated by haploinsufficient or hypomorphic genetic lesions in the perforin pathway[13]. These same patients are at risk for developing rHPS given the appropriate triggers as well.

Importantly, not all patients with rHPS demonstrate defects in cytotoxic killing, suggesting other mechanisms can lead to cytokine storm and HPS as well. For instance, in mice, excessive Toll-like receptor 9 stimulation can lead to a perforin-independent — and indeed lymphocyte-independent — cytokine-driven HPS[16]. Given the clinical overlap between many features of VHF and rHPS, it is intriguing to speculate whether similar pathophysiology is at play at least in part in VHF. This might be conceived as occurring as a result of excessive immune activation, as in the Toll-like receptor 9 model, or perhaps due to a subset of patients with insufficient cytotoxic cell activity, leading to viremia and resultant cytokine storm, suggested for some systemic juvenile idiopathic arthritis patients with MAS[12].

Implications for Treatment of VHF as rHPS

Traditional treatment protocols for familial HLH presenting during infancy differ from management options for rHPS, and include etoposide-based chemotherapy, followed by bone marrow transplantation. This has lowered the 5-year mortality rate from 100% to about 45%. By comparison, in general, therapy for children with rHPS includes a less toxic immunosuppressive/immunomodulatory approach, frequently with high-dose corticosteroids and cyclosporine. The therapeutic guidelines in adults with rHPS who have other comorbidities remain less well defined. More recently, the development of biologic therapeutics targeting proinflammatory cytokines has allowed for additional treatment options in treating rHPS. Novel approaches to treating rHPS include targeting of tumor necrosis factor, IL-1, and IL-6 pathways[12,17]. The most well-reported biologic agent treatment responses for rHPS focus on the use of anakinra, a recombinant human IL-1 receptor antagonist (IL-1Ra)[12].

It is well known in the sepsis literature that the host immune response rather than the pathogen is largely responsible for MODS[18]. Nevertheless, immunosuppressive treatment paradigms for familial HLH and rHPS struggle with the fact that infection is often a trigger of disease, as well as a comorbidity of treatment. Immune modulation is often required in the face of infection to prevent mortality from the primary immune dysfunction. In the case of Dengue virus-induced rHPS, there are multiple reports of corticosteroids and intravenous immunoglobulin (IVIG) used to successfully walk the fine line between infection and immune dysfunction[19,20]. Similarly, 12 patients with Crimean-Congo hemorrhagic fever were successfully treated with high-dose corticosteroids and IVIG[21]. Treating VHF as rHPS strikes at the epitome of this balance, particularly considering that Ebola virus causes a suppression of interferon-induced immunity through viral protein 24 binding to STAT1 and antagonizing interferon signaling[22].

Whether Ebola virus infection will respond to immunosuppression like other VHF viruses, however, is less clear. For example, massive lymphocyte apoptosis has been reported during Ebola infection, questioning the potential benefits of further suppressing the lymphocyte population[23]. There are also data reporting increased proinflammatory cytokine levels in asymptomatic compared to symptomatic Ebola-infected individuals[24], as well as fatal cases of Ebola having lower levels of IL-1 compared to survivors[25]. In this same report, antiinflammatory mediators, such as IL-1Ra and IL-10, were associated with poor prognosis[25]. Nevertheless, it is possible that the increased serum levels of IL-1RA and IL-10 reflect the immune system's attempt to dampen the overexuberant immune response in the sickest Ebola-infected patients experiencing MODS. Indeed, blockade of IL-10 was required to demonstrate hemophagocytosis in a murine model of rHPS[16]. Moreover, the concept of immune suppression of Ebola VHF has been recently considered by others[26].

In considering VHF as a viral infection complicated by rHPS, prudent choice of immune modulation would be of utmost importance. Borrowing from the rHPS experience, IL-1 blockade is emerging as an efficacious intervention that has not demonstrated any increased infectious risk or mortality in clinical trials of sepsis[27]. Although the beneficial role of anticytokine therapy targeting IL-1 in rHPS has yet to be formally validated in clinical trials, the efficacy and safety of this intervention in a disorder associated with up to 60% reported mortality has nonetheless become increasingly recognized[28]. There is now sufficient clinical and laboratory evidence that VHF may reflect viral-induced rHPS. It therefore follows that using a targeted, low infectious risk immunomodulatory approach, such as short term IL-1 blockade and/or IVIG and corticosteroids, might be an unrecognized opportunity to alter the outcome of this disease[26]. Indeed, use of immunomodulatory versus aggressive chemotherapeutic dosing was associated with increased survival in a large cohort of rHPS patients[29]. Secondary consideration of the potential use of other immunomodulatory approaches for rHPS, such as IL-6 blockade or other cytokine pathways that are emerging from other research models, may play a role in our armamentarium as well[17,30].

Given an expanding Ebola epidemic in which mortality nears 70%, a novel approach in management is worthy of investigation, particularly for those individuals who have VHF and features of rHPS. In this case, incorporating treatment of the overly exuberant host immune response to the pathogen as part of the supportive care strategy may provide an opportunity to substantially decrease mortality.

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Ebola virus disease has features of hemophagocytic lymphohistiocytosis syndrome

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The 2014 epidemic of Ebola virus disease (EVD) in several West African countries has mortality rates of up to 70%, as reported by WHO. The exponential increase in the number of infections and deaths, coupled with the absence of specific preventive and therapeutic strategies, represents one of the biggest global health challenges of this millenium. Insights in the pathophysiology and treatment of EVD are thus urgently needed. In this paper, we postulate that EVD has the characteristics of hemophagocytic lymphohistiocytosis (HLH) syndrome (syn., macrophage activation syndrome). The identification of HLH as part of severe EVD, however, brings the possibility for a pathophysiologically targeted approach of therapy; special promise is represented by cytokine-directed therapy in the form of recombinant interleukin-1 receptor antagonist (anakinra). Treatment with anakinra has the advantage to interrupt the deleterious IL-1-mediated hyperinflammatory loop in macrophage activation syndrome, and it has been shown to be associated with remarkable effectiveness and an excellent safety record.

CLINICAL SYNDROME

After an incubation period of 2–21 days, the symptoms of EVD present that resemble flu, including chills, fever, myalgia, and general malaise. As the disease progresses, abdominal complaints such as anorexia, nausea, vomiting, abdominal pain, diarrhea, as well lethargy, headache, and hypotension develop. Also bleeding may develop in 30–50% of patients, however, the severity varies. Bleeding is not *per se* associated with fatal outcome. In severe and fatal forms, death occurs between 6 and 16 days after symptoms appeared because of shock, seizures, severe metabolic complications, and bleeding. In lethal cases, a high blood viral load is seen, accompanied by a significant increment in blood neutrophils, drop in numbers of lymphocytes and platelets, and coagulation abnormalities (1).

Laboratory findings include initially leukopenia (which may drop to 1000 cells/μL) with lymphopenia, while neutrophilia develops thereafter with a left shift with atypical lymphocytes, thrombocytopenia (50000–100000 cells/μL), elevated liver functions with higher aspartate aminotransferase levels compared to alanine aminotransferase, hyperproteinemia, and proteinuria. Diffuse intravascular coagulopathy may be present because of prolonged prothrombin and partial thromboplastin times and the presence of fibrin split products. When a secondary bacterial infection eventually may develop, elevated numbers of white blood cells are documented. In fatal cases, a high blood viral load is seen, accompanied by a significant rise in blood neutrophil count, a progressive drop in lymphocyte and platelet counts, and diffuse coagulation abnormalities (1).

PATHOGENESIS OF EBOLA VIRUS DISEASES

Little is known about the pathology and pathogenesis of EVD in humans. Ebola virus has a broad cell tropism infecting not only immune cells like monocytes, macrophages, and dendritic cells but also various epithelial cells as well as endothelial cells, fibroblasts, hepatocytes, and adrenal cortical cells. Data from experimentally infected non-human primates using Zaire Ebola virus suggest that monocytes, macrophages, and dendritic cells are probably preferably infected at first, whereby these cells subsequently play a significant role to disseminate the virus by spreading it from the side of infection to draining lymph nodes and then to liver and spleen. The tropism of Ebola virus for lymphoid tissue, the liver, and the adrenal gland is important for the pathogenesis as adreno-cortical dysfunction by Ebola virus infection could contribute to the development of shock that characterizes the late stage of disease.

Studies in non-human primates and lethal human EVD show significant lymphocyte reduction and necrosis in spleen, thymus, and lymph nodes, although a significant cellular inflammatory reaction is lacking in these or other infected tissues. The apoptosis that is generally noticed in lymphocytes in infected humans and in non-human primates may clarify the increasing peripheral lymphopenia and tissue lymphoid depletion at post-mortem studies. T-lymphocyte and natural-killer cell populations are mostly reduced in macaques infected with Zaire Ebola virus (2) while human studies indicate that the number of NK cells generally decrease while fatal cases are characterized by especially reduced T-cells counts (3). In addition, blood D-dimer levels can be found in EVD, but are fourfold higher in non-survivors, indicating disseminated intravascular coagulation (DIC) as an important component of severe EVD (4).

HOST IMMUNE RESPONSE DURING EVD

A substantial release of pro-inflammatory mediators and vasoactive elements has

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been shown in patients with severe EVD, contributing to inflammation and coagulation (5). In addition, an ineffective host response causes a high viral load that contributes to a pro-inflammatory state. The release of pro-inflammatory cytokines by monocytes and macrophages is deleterious by causing endothelial leakage, hypotension, DIC, and multiorgan failure, hence a syndrome that closely resembles septic shock. A recent study using 86 samples from the 2000–2001 Uganda outbreak correlated inflammatory biomarkers with clinical outcome, and found that death was associated with elevated concentrations of cytokines (IL-1α, IL-1RA, IL-6, MCP-1, MCSF, MIP-1α) and elevated D-dimer levels, as was found by others. Interestingly, this study also found elevated thrombomodulin and ferritin concentrations, being associated with hemorrhage and death (6).

Host immune responses have also been studied in non-human primates, the current gold standard experimental model of EVD. Strongly up-regulated cytokines/chemokines, including IL-1α, IL-1Ra, IL-6, MCP-1, MIP-1α, IL-15, and IL-18 were reported in experimentally infected macaques (7). Another striking finding was that, although initially fibrinogen levels increase, in the terminal stages of the infection plasma fibrinogen levels were found to decrease rapidly. In humans, plasma fibrinogen levels are generally low, as diffuse intravascular coagulation is very common (1).

HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

Hemophagocytic lymphohistiocytosis (also called macrophage activation syndrome) is an often fatal syndrome of exaggerated but ineffective inflammatory response, characterized by excessive macrophage and T-cell activation and an impaired capacity of NK cells and cytotoxic T cells to kill target cells (8). There are eight diagnostic criteria including fever, splenomegaly, cytopenias affecting at least two of the three lineages in the peripheral blood, hyperferritinemia >5000 μg/L, hypertriglyceridemia or hypofibrinogenemia, hemophagocytosis in the bone marrow, spleen, or lymph nodes, low or absent NK cell activity determined by 51-Cr release assay, and high levels of sCD25 (soluble interleukin-2 receptor). Five of these eight diagnostic criteria are required for diagnosis. Both familial (genetic due to mutations in the perforin pathway) (9) and acquired (reactive) forms of the syndrome are recognized, the latter being triggered by infectious, autoimmune, or neoplastic conditions. Most cases of virus-induced HLH are due to viruses of the herpes family, although a number of other viruses, including parvovirus B19, measles, mumps, influenza, enterovirus, and dengue have also been reported in association with HLH. The key event in HLH is a "cytokine storm" resulting in pancytopenia and hypertriglyceridemia. In addition, the macrophage activation leads to sustained and uncontrolled macrophage and histiocyte infiltration, resulting in hepatosplenomegaly, release of plasminogen activator promoting hypofibrinogenemia, up-regulation of hemoxygenase, and increase in ferritin levels.

HYPOTHESIS: SEVERE EVD IS CHARACTERIZED BY OVERWHELMING MACROPHAGE ACTIVATION SYNDROME

Based on the clinical and pathophysiological characteristics of EVD, we hypothesize that infection with Ebola virus is associated with overwhelming macrophage activation in the setting of a secondary HLH. Strong arguments for this hypothesis are the presence of fever, cytopenia, hypofibrinogenemia, low NK cells, and strikingly elevated ferritin levels, which are also commonly found in EVD, especially in the patients with a fatal outcome. In addition, less perforin mRNA expression was found in peripheral blood mononuclear cells of patients with fatal Ebola infection compared to those that survived (10), while animal studies indicated that CD8-mediated protection against Ebola is perforin-dependent (11).

Cytopenias are key laboratory markers of HLH and are attributed to severe cytokine-mediated inflammation (12). Patients with Ebola infection are often leucopenic at presentation, with abnormal low numbers of lymphocytes and an increased percentage of granulocytes (13). Interestingly, total leukocyte numbers abnormally increase with a rise in immature granulocytes and atypical lymphocytes while the disease develops: this may mirror hyperactivation of bone marrow by cytokines of the IL-1 family that have been proposed to mediate the systemic hyperinflammatory reaction in HLH (14). Thrombocytopenia is a constant feature of EVD, with bone-marrow studies suggesting that thrombocytopenia is not due to reduced platelet production but rather to diffuse intravascular coagulation, as has also been reported in 40–90% of HLH cases (12).

A striking recent finding is the hyperferritinemia in Ebola patients, with mean values around 10,000 μg/L in non-survivors, and 2,500 μg/L in non-fatal cases at day 6–10 after symptom onset. Although hyperferritinemia can be encountered in infections, autoimmune diseases, and several rare syndromes, such very high ferritin concentrations of more than 10,000 μg/L indicate HLH with a sensitivity of 90% and specificity of 96% (ref).

There are also characteristics of EVD that might argue against a classical HLH syndrome. Histiocytic infiltrates of various organs, including liver and spleen, are to be expected in HLH; however, a recent review of human pathology studies indicates that only minimal inflammation is observed in infected tissue and organs in fatal cases, despite high viral loads and necrotic lesions (5). However, Kupffer cell hyperplasia is seen in Ebola infections, similar to HLH (15). Furthermore, no hemophagocytosis has been reported to date in EVD, although very few (if any) studies have been done in this respect. Moreover, the hemophagocytic activity might not occur at all stages of the disease (12) and therefore does not have to be present to diagnose HLH (8).

CONCLUSION AND FUTURE PERSPECTIVES

In conclusion, the clinical manifestations and pathophysiology of severe EVD lead us to propose that HLH characterized by overwhelming macrophage activation is a key event in the pathogenesis of the Ebola infection.

It has been proposed that sepsis, systemic inflammatory response syndrome, multiple organ dysfunction syndrome, and HLH form a disease continuum that share a common mechanism: systemic immune dysregulation triggered by a specific external agent (12). We think that these conditions occur in Ebola virus infection. The metabolic and immune imbalance during EVD requires both optimal support

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measures and the removal of the trigger (infection) that stimulate inflammation. Unfortunately, the latter is not feasible, because no antiviral medication has been of proven efficacy in EVD.

The identification of HLH as part of severe EVD, however, brings the possibility for a pathophysiologically targeted approach of therapy, using the arsenal of immunomodulatory medication known to be successful in HLH. While plasma exchange, corticosteroids, and even immunosuppressive therapy have been proposed for treatment of HLH, special promise is represented by cytokine-directed therapy in the form of recombinant interleukin-1 receptor antagonist (anakinra). Treatment with anakinra has the advantage to interrupt the deleterious IL-1-mediated hyperinflammatory loop in macrophage activation syndrome, and it has been shown to be associated with remarkable effectiveness (16–20). Moreover, the excellent safety record of anakinra argues for its compassionate use in patients with severe EVD in humans.

AUTHOR CONTRIBUTIONS

All authors contributed substantially to the present work, revised the work critically, approved the final paper, and agreed to be accountable for all aspects of the work.

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MAJOR ARTICLE

Ebola Hemorrhagic Fever: Novel Biomarker Correlates of Clinical Outcome

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Background. Ebola hemorrhagic fever (EHF) outbreaks occur sporadically in Africa and result in high rates of death. The 2000–2001 outbreak of Sudan virus–associated EHF in the Gulu district of Uganda led to 425 cases, of which 216 were laboratory confirmed, making it the largest EHF outbreak on record. Serum specimens from this outbreak had been preserved in liquid nitrogen from the time of collection and were available for analysis.

Methods. Available samples were tested using a series of multiplex assays to measure the concentrations of 55 biomarkers. The data were analyzed to identify statistically significant associations between the tested biomarkers and hemorrhagic manifestations, viremia, and/or death.

Results. Death, hemorrhage, and viremia were independently associated with elevated levels of several chemokines and cytokines. Death and hemorrhage were associated with elevated thrombomodulin and ferritin levels. Hemorrhage was also associated with elevated levels of soluble intracellular adhesion molecule. Viremia was independently associated with elevated levels of tissue factor and tissue plasminogen activator. Finally, samples from nonfatal cases had higher levels of sCD40L.

Conclusions. These novel associations provide a better understanding of EHF pathophysiology and a starting point for researching new potential targets for therapeutic interventions.

Keywords. Ebola virus; biomarkers; hemorrhage; Gulu; hemorrhagic fever virus.

Ebola hemorrhagic fever (EHF) was first identified in 1976 during an outbreak in the Democratic Republic of the Congo (formerly known as Zaire). Since then, 21 additional outbreaks among humans have been recorded in sub-Saharan Africa. The largest of these outbreaks took place in the Gulu district of Uganda in 2000–2001 and was caused by Sudan virus (SUDV). This outbreak resulted in 425 cases, 216 of which were laboratory confirmed, and a 53% overall case-fatality rate [1].

The genus *Ebolavirus* includes 5 different viruses: SUDV, Tai Forest virus (TAFV), Reston virus (RESTV), Ebola virus (EBOV), and Bundibugyo virus (BDBV). EHF caused by EBOV has the highest fatality (57%–90%), followed by SUDV (41%–65%) and BDBV (40%); to date, RESTV infection has been asymptomatic in humans, and TAFV has only been identified in 2 human infections, both of which were nonfatal [2–4]. Clinical EHF is characterized by abrupt onset of fever, fatigue, headache, myalgia, and gastrointestinal distress 3–13 days after exposure to virus [5]. Many patients develop hemorrhagic manifestations, which has led to the term "hemorrhagic fever."

The samples collected during the Gulu outbreak have already yielded extensive information about EHF pathophysiology, including the observation that aspartate aminotransferase (AST), D-dimer, blood urea nitrogen (BUN), and creatinine levels are higher than normal, while calcium and albumin levels are lower than normal in samples from fatal EHF cases [6]. Elevated levels of the cytokines interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 10 (IL-10), and macrophage inflammatory protein 1β (MIP-1β) were also associated with fatal outcomes [7]. Additionally, human leukocyte antigen B67 (HLA-B67), HLA-B15, and marked CD8

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lymphopenia were associated with fatal outcome, and HLA-B7 and HLA-B14 were associated with nonfatal outcome [8, 9].

The goal of our study was to better understand the pathophysiology of EHF by correlating hemorrhagic manifestations and death with specific biomarkers. We measured levels of markers of immune function, endothelial activation, and coagulation in available patient serum samples, to determine whether alterations in levels of these biomarkers were associated with hemorrhagic manifestations, viremia, or death.

METHODS

Study Design

During the 2000–2001 Gulu outbreak, an international response team, including Centers for Disease Control and Prevention (CDC) staff, responded with clinical and technical assistance. Serum samples were frozen in liquid nitrogen in the field and have been stored that way since the time of the outbreak. For our analyses, samples were chosen to represent sex ratios, hemorrhagic manifestations, and death rates consistent with those observed during the outbreak (Table 1). Specimens were prioritized for novel analyses; serum chemistry analyses were performed on samples of sufficient volume. All samples were inactivated by γ irradiation before use.

Institutional Review Board (IRB)

IRB approval was obtained before initiating the study, and an exemption was granted by the CDC Human Research Protection Office.

Luminex-Based Assays

The following assays were purchased from Affymetrix (Santa Clara, CA) and performed according to the manufacturer's instructions: a 26-plex assay for granulocyte macrophage colony stimulating factor (GM-CSF), GROα, interferon α2 (IFN-α2), IFN-β, IFN-γ, IL-10, interleukin 12p70 (IL-12p70), IL-12p40, interleukin 1α (IL-1α), IL-1β, IL-1 receptor antagonist (IL-1RA), interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 5 (IL-5), IL-6, IL-8, IFN-γ–inducible protein 10 (IP-10), monocyte chemoattractant protein 1 (MCP-1), macrophage colony-stimulating factor (MCSF), MIP-1α, MIP-1β, soluble CD40 ligand (sCD40L), soluble E-selectin (sE-selectin), soluble Fas ligand (sFasL), tumor necrosis factor α (TNF-α), and vascular endothelial growth factor A (VEGF-A); a 2-plex assay for D-dimer and tissue plasminogen activator (TPA); a 5-plex assay for plasminogen activator inhibitor-1 (PAI-1), serum amyloid antigen (SAA), regulated on activation, normal T-cell expressed and secreted (RANTES), soluble intracellular adhesion molecule 1 (sICAM-1), and soluble vascular cell adhesion molecule (sVCAM-1); and single-plex assays for C reactive protein (CRP) and fibrinogen. Assays for ferritin and cortisol were performed as single-plex assays according to manufacturer's instructions (Millipore, Billerica, MA). 2-plex assays were performed for tissue factor (TF) and thrombomodulin according to the manufacturer's instructions (Millipore). For samples with values outside the upper end of the standard curve, additional dilutions were made as necessary to obtain accurate values for all analytes.

Table 1. Patient Characteristics

Characteristic	This Study (n = 86)	Gulu Outbreak (n = 425)
Age, y, range	6–60	0.25–81
Sex		
Female	56 (65)	267 (63)
Male	30 (35)	158 (37)
Any hemorrhagic manifestation	31 (36)	128 (30)
Death	41 (48)	224 (53)

Data are no. (%) of patients, unless otherwise indicated. Gulu outbreak data are from Okware et al [1].

Enzyme-Linked Immunosorbent Assays (ELISAs)

Mannose-binding lectin (MBL; Hycult Biotech, Plymouth Meeting, PA) and total immunoglobulin G (IgG; eBioscience, San Diego, CA) ELISAs were performed according to the manufacturers' instructions. For samples with values outside the upper end of the standard curve, additional dilutions were made as necessary to obtain accurate values for all analytes.

Serum Chemistry Analyses

A Piccolo comprehensive metabolic reagent disc was run on the Piccolo xpress Chemistry Analyzer (Abaxis, Union City, CA) to determine serum chemistry values for alanine aminotransferase (ALT), albumin, AST, alkaline phosphatase, calcium, chloride, creatinine, glucose, potassium, sodium, total bilirubin, total CO_2, total protein, and BUN. Only 132 of the 187 samples had sufficient volume for serum chemistry analyses.

Human Immunodeficiency Virus (HIV) Testing

The Multispot HIV-1/HIV-2 Rapid Test (BioRad, Hercules, CA) was used to assess the HIV status of the patient cohort. Two patients (ages 7 and 19 years) did not have sufficient sample for analysis.

Viremia Analysis via Measurement of RNA Copy Number

Total RNA was isolated from serum using the MagMAX-96 Viral RNA Isolation Kit on the MagMAX Express-96 Magnetic Particle Processor (Ambion, Grand Island, NY). In parallel, serial dilutions of a normal human serum specimen inoculated with a known titer of SUDV was subjected to RNA isolation and used to generate a standard curve. Real-time polymerase chain reaction was then performed as previously described [10].

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Statistical Analysis

Data on sex, age, date of symptom onset, viremia levels, hemorrhagic manifestations, HIV status, patient survivability, and date of sample collection were available for the 86 patients included in our study. Only samples taken 0–15 days from symptom onset were analyzed, to represent the normal course of the illness.

Analysis of variance (ANOVA) was conducted for each of the 55 analytes, using sex, age, day from symptom onset, viremia, death, hemorrhaging, and HIV status as the independent variables. All variables were converted to categorical variables (meaning that there was limited number of outcomes [ie, viremic vs not viremic]) because of the number of samples and the distribution of the numerical data, with the date from symptom onset converted to 3 categories (0–5 days, 6–10 days, and 11–15 days). An initial overall α of 0.25 was set, and the Bonferroni inequality was used to arrive at a critical P value of .0045 for the individual analytes. Using these criteria, 29 of the 55 analytes had statistically significant differences. Knowing the impact of multiple testing on the power of tests, the technique of Yoav and Hochbergs was used to control the false discovery rate to balance the power and type 1 error of the analyses [11]; this correction resulted in 10 additional analytes meeting the criteria for statistical significance. The independent variables were analyzed to measure colinearity, but no significant variance inflation was observed.

Ultimately, our study included 161 samples, each described by 9 categorical variables. Each sample was analyzed for 41 different analytes (one sample was insufficient to perform a subsequent analysis of 2 analytes). An analysis of metabolic information was attempted for all samples, but only 132 samples had sufficient volume for analyzing 14 additional analytes.

Eight analytes (IL-12p70, IL-12p40, IL-2, IL-4, IL-5, sFasL, IFN-γ, and IFN-α2) had statistically significant correlations but were excluded from analysis because $\geq$30% of the samples had a value below the limit of detection. We therefore did not attempt to make conclusions based on data that could have been affected by degradation over time.

Model Selection

After the initial ANOVA, 27 significant analytes were selected for further analysis. Stepwise regression for each significant analyte was used to find the best model, excluding insignificant variables at an α of 0.05. Particular attention was paid to those analytes in which age, death, viremia, hemorrhaging, and HIV status were significant. Ten analytes were significant for age, 16 for death, 7 for viremia, 8 for hemorrhaging, and 2 for HIV status. We compiled the analytes that were significantly different for these variables and calculated the means and standard error for each interval.

Finally, 2 additional statistical tests were performed to measure the relationships between death and both viremia levels and the presence of hemorrhaging. The distribution of the viremia levels was clearly nonnormal, so the Mann–Whitney U test was used to determine differences in viremia for patients with fatal and those with nonfatal outcomes. Patients with no measurable viremia were excluded from analysis. The survivability of patients with and those without hemorrhaging was also examined, but no significant differences were found.

RESULTS

Study Population

The study included 86 EHF patients from the 2000–2001 Gulu outbreak that were representative of the outbreak as a whole. As noted in previous outbreaks, more women were affected than men, likely because women are commonly caretakers, a known risk factor for contracting EHF. Thirty-six percent of the patients in this study had hemorrhagic manifestations (defined as any of the following: vomiting blood, blood in the stool, or bleeding from the gums, skin, or eyes), and approximately half of the patients died, consistent with reported death rates for SUDV (Table 1). One limitation to our study was that the limited sample volume influenced our selection of samples because we only selected patients for whom there were sufficient sample volume to complete the desired testing. This may have biased our data slightly toward the nonfatal group.

In the filovirus field, it has generally been assumed that immune system deficiencies associated with HIV infection would likely negatively impact EHF outcome. As the HIV status of EHF patients has never been determined previously, HIV testing was conducted on patients included in this study. None of the patients <18 years of age were HIV positive. Of the 67 adults (ages 18–60 years), 12 (17.9%) were HIV positive. This rate is roughly consistent with data on HIV prevalence in the Gulu region of Uganda during the time of the outbreak; 12% of women attending antenatal clinics were reported to be HIV positive [12]. Of the 12 HIV-positive adults, 5 were male and 7 were female. Although $CD4^+$ T-cell counts were unknown in these patients, 50% of the HIV-positive adults (3 males and 3 females) had fatal outcomes. This mortality rate was approximately the same as that in the total adult population, suggesting that an individual's HIV status did not significantly affect outcome during SUDV infection.

Study Design

Fifty-five different biomarkers were assessed in 187 serum samples in this study. Thirty-six patients had $\geq$2 serial samples collected 1–15 days after onset of symptoms; composite data rather than individual patient data are presented, to provide the most objective data for analysis. Supplementary Table 1 provides the statistical significance for each biomarker tested and its relationship to the examined variables. The data presented in this article represent the statistically significant associations observed

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Table 2. Coefficient of Variance (CV) Between Repeat Chemistry Measurements After 12 Years of Storage in Liquid Nitrogen

CV	ALT	AST	BUN	CRE	TP	ALB
Percentage difference, mean (range)	2.3 (0–7.4)	1.5 (0–4.4)	1.7 (0–7.1)	2.2 (0–5.4)	0.96 (0–2.9)	0.75 (0–2.8)

Abbreviations: ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CRE, creatinine; TP, total protein.

between a given biomarker and death, hemorrhagic manifestations, or viremia.

Serum Chemistry Analysis and Viremia

Serum chemistry analyses were performed on many patients as part of their clinical management during the 2000–2001 outbreak. These data were still available, allowing for a direct assessment of the stability of several metabolic analytes after 12 years of storage in liquid nitrogen. Many analytes were strikingly stable, as shown by a maximal coefficient of variance of 7.4% between the original measurement and the repeat measurement for ALT, AST, BUN, creatinine, total protein, and albumin levels (Table 2). The more labile components, CO_2 and electrolytes, were excluded from our analysis.

Previously reported associations between fatal outcomes and AST, BUN, creatinine, and albumin levels [6] were again seen in our study (data not shown) [6]. Higher levels of viremia were associated with a fatal outcome, as previously demonstrated [10] (Figure 1).

Cytokines and Chemokines

Cytokines and chemokines are a diverse, extensively studied group of proteins that modulate the immune response. We analyzed 25 different cytokines and chemokines. IL-1α, IL-1RA, IL-6, MCP-1, MCSF, and MIP-1α levels were all elevated in patients with a fatal outcome (Figure 2*A*–*E* and 2*G*).

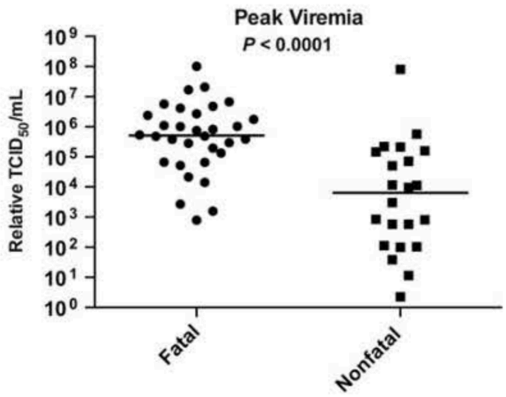

Figure 1. Viremia as a function of clinical outcome. Patients with fatal outcomes had higher viral loads. $TCID_{50}$, median tissue-culture infective dose.

Previously published data have shown associations between MIP-1β, IL-6, IL-8, and IL-10 levels and fatal outcomes in SUDV-infected patients [7]. All of these prior associations were observed in our study. However, IL-8 levels were only elevated very late in the infection in patients with a fatal outcome (data not shown); MIP-1β levels tended to be higher in patients with fatal outcomes but did not meet the cutoff for statistical significance; and IL-10 levels demonstrated an age-specific association with death. GRO-α levels, similar to IL-8 levels, were only elevated late during infection in patients who died (data not shown). IL-1α, MCSF, and IP-10 levels were elevated in samples with documented viremia, compared with samples with no detectable virus, but this finding was independent of their relationship with death (data not shown). MCSF, MIP-1α, and IP-10 levels were also elevated in patients who developed hemorrhagic manifestations (Figure 2*F*, 2*H*, and 2*I*). Unexpectedly, elevated levels of sCD40L correlated with nonfatal outcomes, a first for any report of EHF (Figure 2*J*).

Biomarkers of Inflammation

The acute phase response refers to a constellation of host responses that occur following infection and in other inflammatory diseases [13]. These responses are classically triggered by proinflammatory cytokines, such as IL-6, IL-1, IFN-γ, and TNF-α. Increased liver production of CRP and SAA and decreased production of albumin are common consequences of the acute-phase reaction. In addition, hypergammaglobulinemia and hyperferritinemia are often seen. These markers of inflammation are used clinically in many disease processes to help in diagnosis and to follow a patient's response to therapy. CRP levels were within the normal range in all samples and were excluded from further analysis. Ferritin levels were elevated in patients with fatal outcomes and in those with hemorrhagic manifestations (Figure 3*A* and 3*B*). Ferritin levels were also associated with viremia, independent of the associations with death and hemorrhage (data not shown). Ferritin levels were higher in HIV-positive patients than in HIV-negative patients (data not shown); this has been previously noted specifically in association with advanced HIV disease [14, 15]. HIV-positive patients also had higher total IgG levels than HIV-negative patients (data not shown), a well-documented phenomenon [16]. Finally, levels of sICAM, a marker of endothelial activation [17], were elevated in patients with hemorrhagic manifestations (Figure 3*C*)

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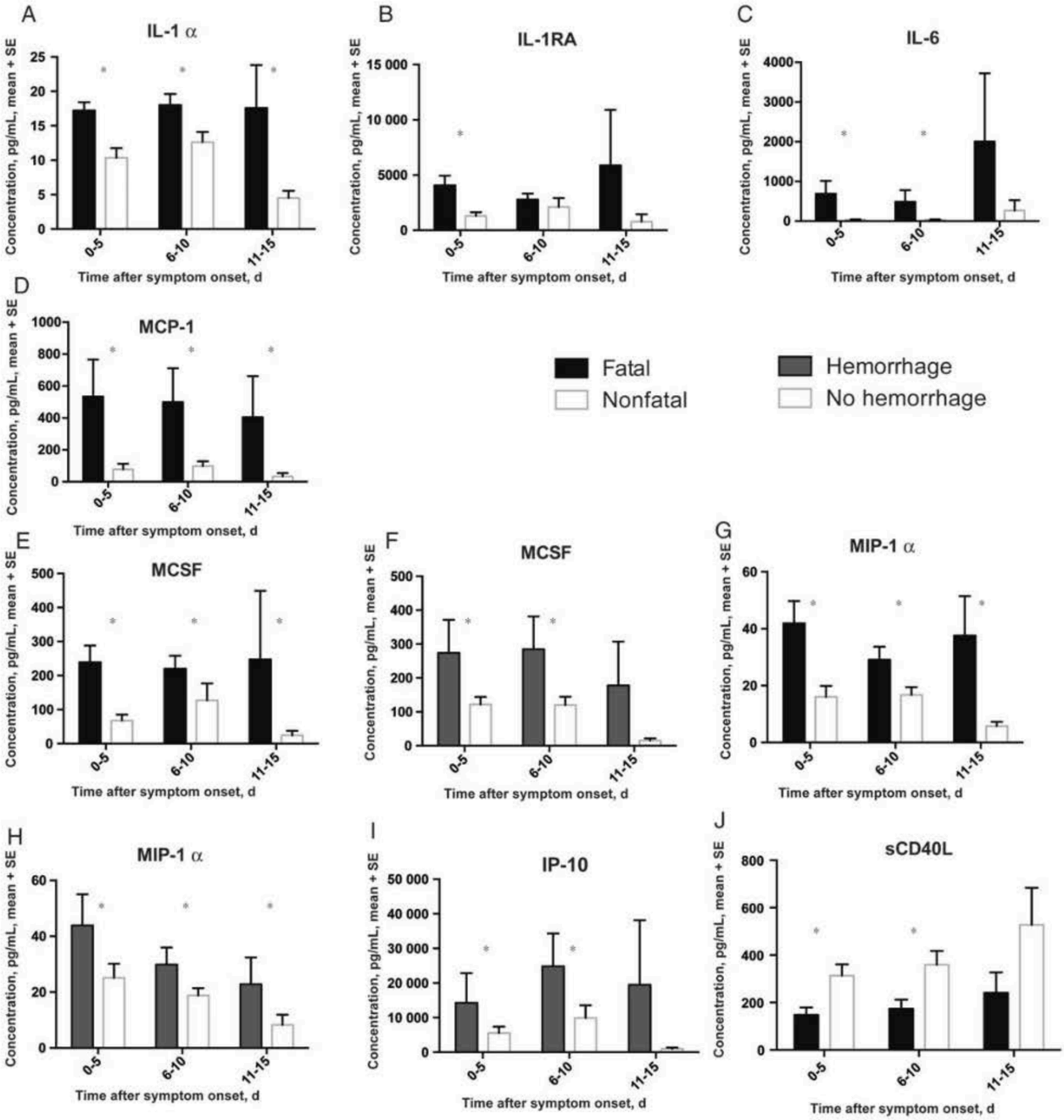

Figure 2. Cytokines and chemokines associated with a fatal outcome (black bars) or hemorrhagic manifestations (red bars). *$P \leq .05$. Abbreviations: IL-1α, interleukin 1α; IL-1RA, interleukin 1 receptor antagonist; IL-6, interleukin 6; IP-10, interferon γ–inducible protein 10; MCP-1, monocyte chemoattractant protein 1; MCSF, macrophage colony-stimulating factor; sCD40L, soluble CD40 ligand; SE, standard error.

Biomarkers of Coagulopathy

The frequency of hemorrhagic manifestations during EHF warranted an examination of the measureable factors that control hemostasis. The delicate balance of coagulation and fibrinolysis is depicted in a simplified model in Figure 4. In this study, PAI-1, fibrinogen, TPA, D-dimer, thrombomodulin, and TF levels were measured in all patient samples. TPA, D-dimer, thrombomodulin, and TF levels were all statistically significant in our analysis. Thrombomodulin levels were elevated in patients with fatal outcomes and in patients with hemorrhagic manifestations (Figure 4*A* and 4*B*). D-dimer levels were elevated in patients with a fatal outcome (Figure 4*C*), as previously reported [6]. TPA levels were higher in viremic patients (Figure 4*D*). TF levels were within the normal range in all samples

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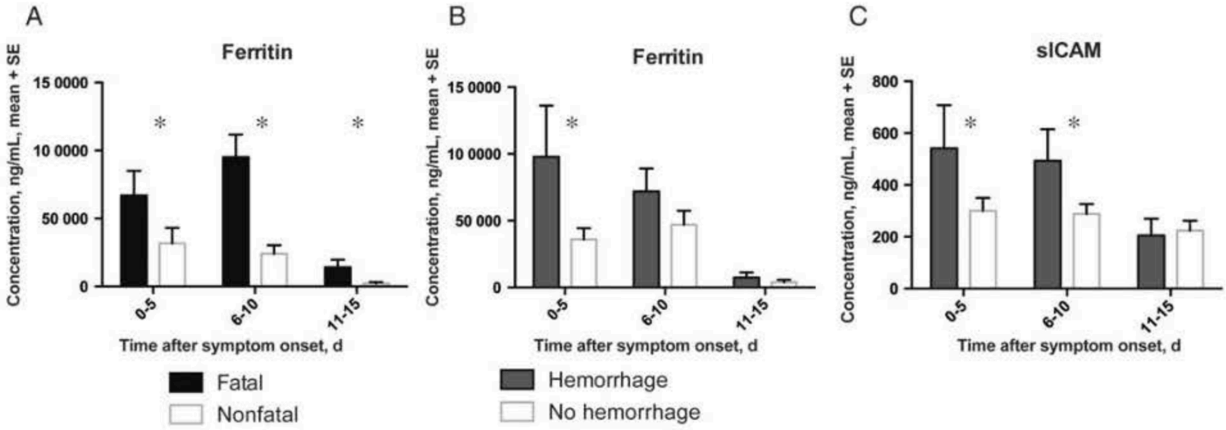

Figure 3. Markers of inflammation associated with a fatal outcome (black bars) or hemorrhagic manifestations (red bars). $^{*}P \leq .05$. Abbreviations: SE, standard error; sICAM, soluble intracellular adhesion molecule 1.

tested but were slightly higher in samples with detectable viremia than in those without detectable viremia (Figure 4*E*). Notably, TF levels were not associated with hemorrhagic manifestations or death.

DISCUSSION

We found associations between multiple proinflammatory cytokines and increased death rates, as previously shown in humans [7, 18–20] and EBOV-infected nonhuman primates [21], consistent with the idea of systemic inflammation contributing to a fatal outcome. We also found several novel associations, most strikingly, the association between increased sCD40L levels and nonfatal outcomes. Since sCD40L is largely produced by activated platelets [22], increased sCD40L levels could represent ongoing repair of altered endothelium by activated platelets. Additionally, nitric oxide (NO) inhibits platelet activation [23], and elevated NO levels have been reported in fatal EHF cases [9]. Therefore, the deleterious effects of NO could be related to inhibiting platelet activation, in addition to its vasodilatory effects. Effective repair of altered endothelium by activated platelets could lead to improved hemodynamic stability in patients and, thus, improved clinical outcomes.

Ferritin, another biomarker we examined, is a well-established acute-phase reactant that has been associated with severe disease in both dengue and Crimean Congo hemorrhagic fevers [24, 25]. Ferritin is the intracellular storage form of iron, and its levels increase during times of stress or inflammation. Ferritin levels were higher in samples from patients with EHF who had fatal outcomes, detectable viremia, and hemorrhagic manifestations; thus, ferritin could prove to be a useful clinical maker of EHF severity.

The striking clinical manifestation of hemorrhage during historic EHF outbreaks led to the naming of the disease as Ebola *hemorrhagic* fever, which has resulted in the perception that hemorrhage occurs in most patients and is associated with fatal outcomes. However, published data from the 3 largest EHF outbreaks, caused by EBOV (Kikwit, Democratic Republic of the Congo, 1995), SUDV (Gulu, Uganda, 2000), and BDBV (Bundibugyo, Uganda, 2007), demonstrate hemorrhagic manifestations in only 41%, 30%, and 46.5% of total cases, respectively [1, 3, 26]. Furthermore, no significant correlation between hemorrhage and death has been found in these same outbreaks of EBOV and BDBV infections. Consistently, we found no statistically significant differences in survival between patients with and those without hemorrhage ($P = .23$). Therefore, it is not surprising that, of the 6 different coagulation and fibrinolysis markers we assessed, only thrombomodulin was associated with both fatal outcome and hemorrhagic manifestations. Thrombomodulin is a marker of endothelial activation/dysfunction and, as discussed below, has many functions besides inhibiting coagulation. There is a common perception, not only in EHF but also in routine sepsis, that correction of coagulopathy will improve survival outcomes. However, therapeutic interventions that have focused on targeting coagulopathy in nonhuman primate models of EBOV only moderately increased survival [27, 28], and use of activated protein C in septic patients in the intensive care unit has been similarly disappointing [29]. A large focus has been placed on understanding the role of TF in EHF, given the marked increases in TF expression seen in monocytes in nonhuman primates infected with EBOV [30]. However, when TF levels were examined in patients from this outbreak, which was caused by SUDV, none of the patients had elevated levels of TF outside of the normal range, and there were no associations between TF levels and death or hemorrhage. These data are limited by our ability to only sample plasma TF versus monocyte-associated TF, although plasma and monocyte TF levels correlated in a human study of

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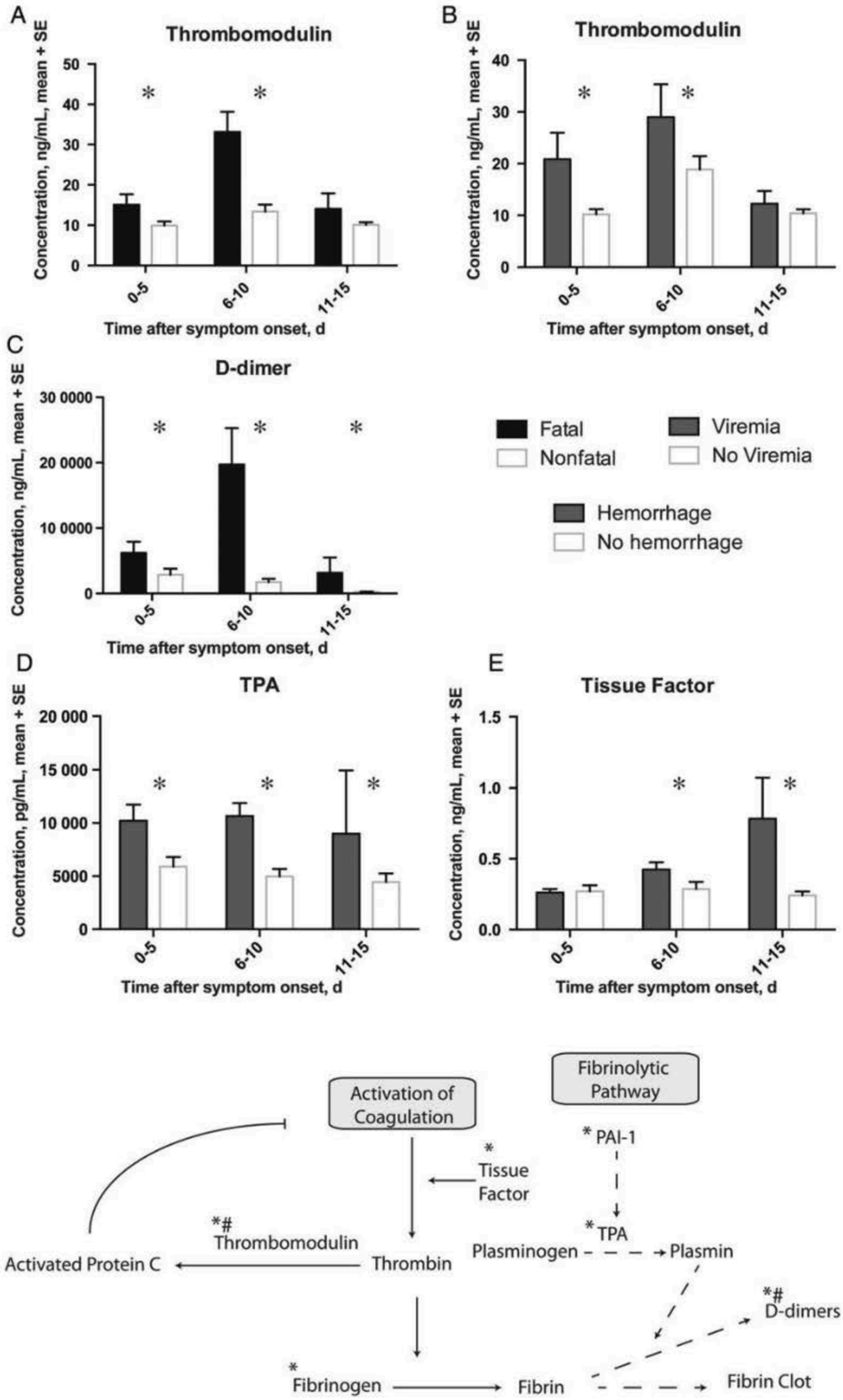

Figure 4. Markers of coagulation and fibrinolytic pathways associated with a fatal outcome (black bars), hemorrhagic manifestations (red bars) or viremia (blue bars). *$P \leq .05$. A simplified model of the coagulation/fibrinolytic pathway is also depicted. Proteins that are known to play a major role in the pathways are noted. Arrows indicate activation while bars indicate inhibition. *, analytes assessed in the study; #, analytes associated with death.

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thrombosis [31]. Finally, the nonhuman primate studies were performed with EBOV, whereas the patients in this study were infected with SUDV, so these TF differences could be virus or host related.

Thrombomodulin is released by an activated endothelium and functions as an anticoagulant by activating protein C when present in the microenvironment on the surface of endothelial cells. In our study, thrombomodulin levels were elevated in samples from patients who died and from those with hemorrhagic manifestations, the latter being consistent with its anticoagulant function. Thrombomodulin has several protein domains that are responsible for its anticoagulant activity, but it also contains a C-type lectin domain that has antiinflammatory activities, including maintaining endothelial integrity [32]. The fact that EBOV GP protein binds to other C-type lectins [33–35] makes the thrombomodulin C-lectin domain an attractive subject for further study. If thrombomodulin's C-lectin domain–mediated functions are inhibited by association with viral GP, this could provide a possible explanation for how these viruses are able to disrupt endothelial function without causing direct cytopathic effects. Alternatively, overactivation of endothelial cells, leading to shedding of thrombomodulin into the plasma, could result in a loss of thrombomodulin's stabilizing function at endothelial junctions, contributing to vascular leakage.

MCSF, MIP-1α, IP-10, sICAM, and thrombomodulin levels were all associated with hemorrhage in our study, and on the basis of findings in the literature, their activities can all be correlated with leukocyte/endothelium interactions. In one possible model, these cytokines could recruit leukocytes to areas of inflammation, and production of adhesion molecules such as ICAM would facilitate leukocyte adhesion, rolling, and diapedesis. Activated endothelial cells would then release thrombomodulin into the bloodstream, so it would no longer be present in the microenvironment to activate protein C and coagulation, nor would it be present to stabilize the endothelium. This would leave an activated, leukocyte-enriched, procoagulant endothelium, causing dysregulated hemostasis, which could manifest clinically as hemorrhage.

Future pathogenesis models could evaluate the possible role of activated platelets in improving clinical outcomes by therapeutic administration of platelets, inhibitors of NO synthetase, or active sCD40L to infected animals. Finally, the potential roles of thrombomodulin C-lectin domains in modulating EHF outcomes should be further studied at the molecular level to determine whether viral glycoproteins interact with thrombomodulin. If this proves to be the case, evaluating the role of thrombomodulin C-lectin domains in animal models would be warranted.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflict of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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    /ENU ()
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<<
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  /PageSize [612.000 792.000]
>> setpagedevice
```

AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Fri, 11 May 2018 19:33:59 +0000
To: Billet, Courtney (NIH/NIAID) [E]
Cc: Folkers, Greg (NIH/NIAID) [E];Conrad, Patricia (NIH/NIAID) [E];Stover, Kathy (NIH/NIAID) [E]
Subject: RE: For your review, HVAD Statement
Attachments: HVAD statement 2018 to ASF - with Fauci tracked changes.docx

See my tracked changes. Thanks.

From: Billet, Courtney (NIH/NIAID) [E]
Sent: Wednesday, May 09, 2018 2:06 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Folkers, Greg (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6) Stover, Kathy (NIH/NIAID) [E] (b)(6)
Subject: ASF: For your review, HVAD Statement

Attached for your review is our draft statement for HIV Vaccine Awareness day (May 18). We still don't know the pub date for Peter Kwong's new antibody finding, so that is noted as pending. We've set this up to be co-authored with Maureen Goodenow, at Carl's suggestion. Apparently the co-authorship means quite a lot to her.

AMERICAN OVERSIGHT

Page 0439 of 2068

Withheld pursuant to exemption

(b)(5)

of the Freedom of Information and Privacy Act

AMERICAN OVERSIGHT

Page 0440 of 2068

Withheld pursuant to exemption

(b)(5)

of the Freedom of Information and Privacy Act

AMERICAN OVERSIGHT

From: Folkers, Greg (NIH/NIAID) [E]
Sent: Fri, 11 May 2018 15:42:55 +0000
To: Fauci, Anthony (NIH/NIAID) [E];(b)(6)
Cc: Folkers, Greg (NIH/NIAID) [E];Conrad, Patricia (NIH/NIAID) [E];Paules, Catharine (NIH/NIAID) [E]
Subject: RE: ASM CVS Keynote Address
Attachments: 2018-05-06 ASM Clinical Virology Symposium West Palm Beach -- influenza asf-niaid.pdf

Ms Boyer,
Thanks for your note to Dr Fauci, who asked that I reply on his behalf.
I have attached his slide set from the ASM CVS meeting.
Thanks for your interest, and best regards.

Greg
Gregory K. Folkers, M.S., M.P.H.
Chief of Staff, Immediate Office of the Director
National Institute of Allergy and Infectious Diseases
National Institutes of Health
9000 Rockville Pike
Bldg 31, Room 7A-05
Bethesda, MD 20892
Tel: (b)(6)
Cell: (b)(6)

From: Boyer, Jennifer (b)(6)
Sent: Thursday, May 10, 2018 12:58 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: ASM CVS Keynote Address

Hi Dr. Fauci,

My name is Jennifer Boyer; I used to work in Microbiology in the Clinical Center at NIH from 2010, under Dr. Murray, until 2016 when I transferred to University of Maryland Medical Center for my current management position in Microbiology. I had the opportunity to attend both the PASCV Molecular Virology Workshop and ASM CVS this year. Your keynote address on Sunday was so very captivating and intriguing, it was brilliant. I was reading over the notes that I took on your presentation; would it be possible to send me your slides or notes by chance? I'm sure that others are asking the same thing as your address was amazing.

Thanks so very much,

AMERICAN OVERSIGHT

Jennifer L. Boyer, BS, M(ASCP)CM
Technical Specialist, Microbiology
University of Maryland Medical Center
22 S. Greene St., Rm N2W50
Baltimore, MD 21201
(b)(6)
(b)(6)

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AMERICAN OVERSIGHT

ASM Clinical Virology Symposium

Universal Influenza Vaccine: Challenges and Opportunities

Anthony S. Fauci, M.D.
Director
National Institute of Allergy and Infectious Diseases
National Institutes of Health

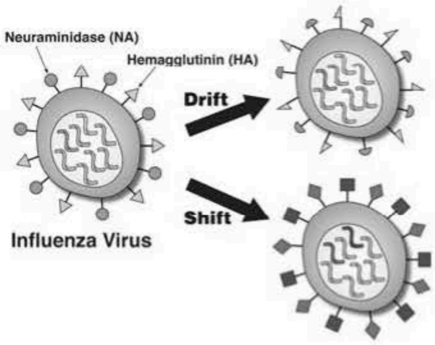
May 6, 2018

Influenza A Viruses

- Single-stranded, segmented RNA viruses
- Present in a wide variety of birds and mammals
- Wild waterfowl are natural hosts
- Can cause serious disease in humans

AS Fauci/NIAID

Seasonal vs. Pandemic Influenza

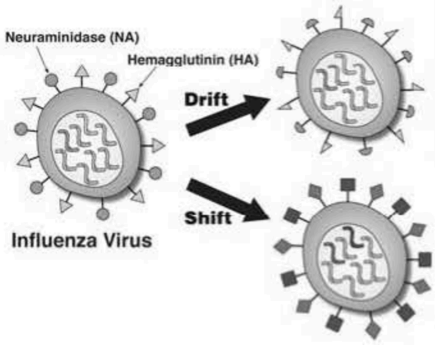


Seasonal Influenza
- Annual occurrence
- Residual immunity in population

Pandemic Influenza
- Unpredictable rare occurrence
- "Naïve" population

AS Fauci/NIAID

Seasonal vs. Pandemic Influenza



Seasonal Influenza
- Annual occurrence
- Residual immunity in population

Pandemic Influenza
- Unpredictable rare occurrence
- "Naïve" population

AS Fauci/NIAID

Significant Annual Burden of Influenza

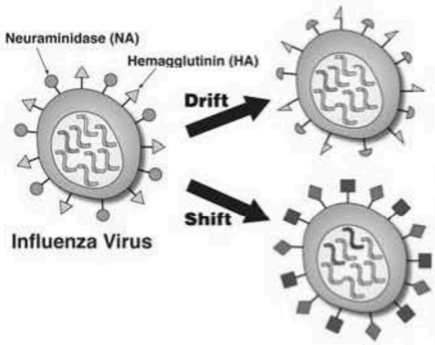
United States		Global
12,000 – 56,000	Deaths	291,000 – 646,000
140,000 – 710,000	Severe Cases Hospitalizations	3M to 5M
9.2M – 35.6M	Cases	1.0B

Direct medical costs: $10.4B per year
Indirect and direct costs: $87.1B per year

AS Fauci/NIAID

Source: CDC, 2018.

Seasonal vs. Pandemic Influenza



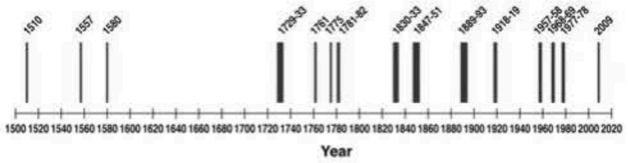
Seasonal Influenza
- Annual occurrence
- Residual immunity in population

Pandemic Influenza
- Unpredictable rare occurrence
- "Naïve" population

AS Fauci/NIAID

AMERICAN OVERSIGHT

500 Years of Likely and Documented Influenza Pandemics



Courtesy of David Morens

AS Fauci/NIAID

Influenza Pandemics in the 20th–21st Centuries

1918	H1N1	"Spanish Flu"	~50 million deaths
1957	H2N2	"Asian Flu"	1-2 million deaths
1968	H3N2	"Hong Kong Flu"	700,000 deaths
2009	H1N1	"Swine Flu"	284,500 deaths

AS Fauci/NIAID

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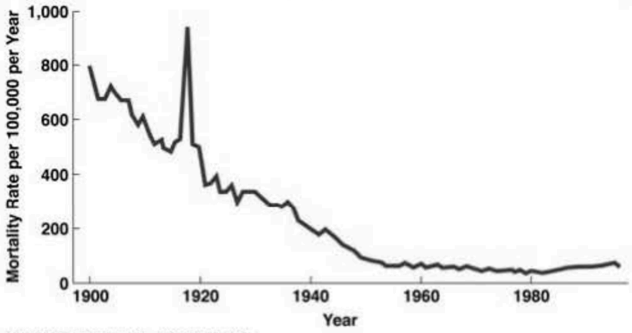
2009	H1N1	"Swine Flu"	284,500 deaths

AS Fauci/NIAID

Crude Infectious Disease Mortality Rate, United States, 1900 to 1996



Source: GL Armstrong et al., *JAMA* 281:61, 1999

AS Fauci/NIAID

- Current seasonal influenza vaccines are not consistently effective
- Pandemics do occur and response after the fact is not effective
- "Chasing after" potential pandemic outbreaks (pre-pandemic viruses) is costly and ineffective

AS Fauci/NIAID

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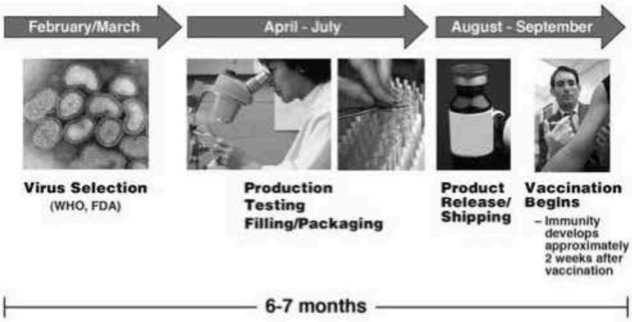
Adjusted Influenza Vaccine Effectiveness Estimates, United States

AS Fauci/NIAID

Antigenic mismatch is a well known cause of decreased seasonal influenza vaccine effectiveness...

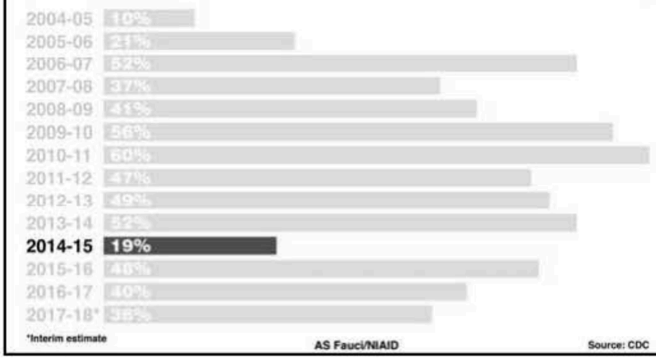
AS Fauci/NIAID

Seasonal Influenza Vaccine Development



AS Fauci/NIAID

Adjusted Influenza Vaccine Effectiveness Estimates, United States



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THE BALTIMORE SUN — December 12, 2014

Flu Vaccine Still Advised Despite 'Drifting' of One Strain of Virus

Pittsburgh Post-Gazette — December 22, 2014

Vaccine Mismatch, Flu Afflict Region

INDEPENDENT — February 6, 2015

Flu Vaccine Given to Millions This Winter is Basically Useless

AS Fauci/NIAID

but what about years where the match is a good one?

AS Fauci/NIAID

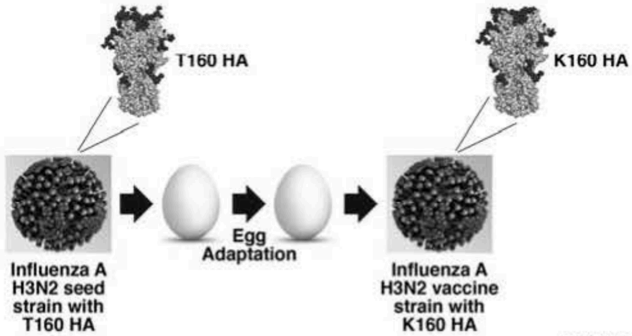
AMERICAN OVERSIGHT

Adjusted Influenza Vaccine Effectiveness Estimates, United States

AS Fauci/NIAID

Source: CDC

Egg Adaptation of Influenza A H3N2 May Contribute to Decreased Vaccine Effectiveness



AS Fauci/NIAID

What happened during the 2017-2018 flu season in the United States?

AS Fauci/NIAID

NBC

December 2, 2017

Flu Is Spreading Fast This Year, with a Severe Season Possible

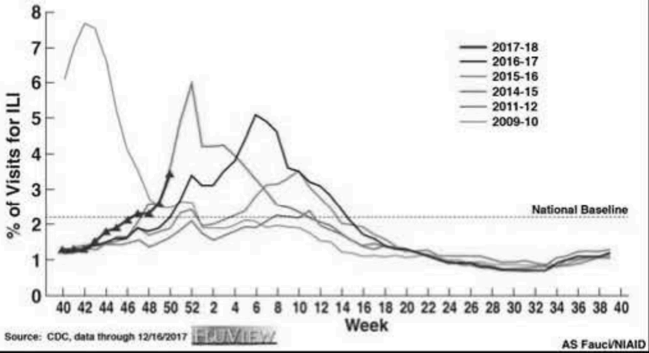
AS Fauci/NIAID

Geographic Spread of Influenza as Assessed by State and Territorial Epidemiologists, January 20, 2018

Source: CDC, week ending 1/20/2018

AS Fauci/NIAID

Weekly Reporting of Influenza-Like Illness in the United States by Season



Source: CDC, data through 12/16/2017

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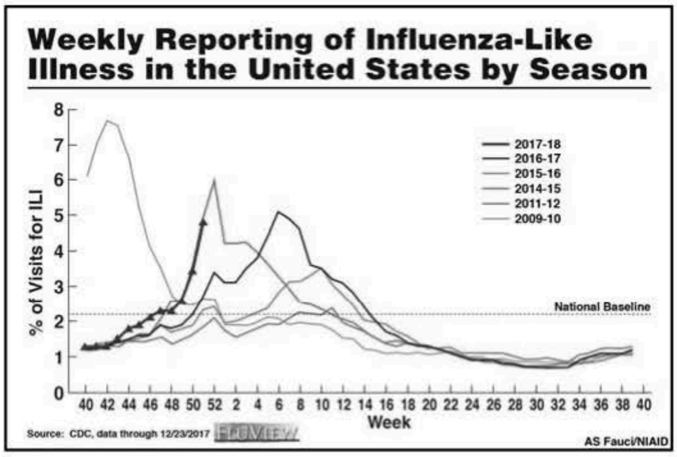

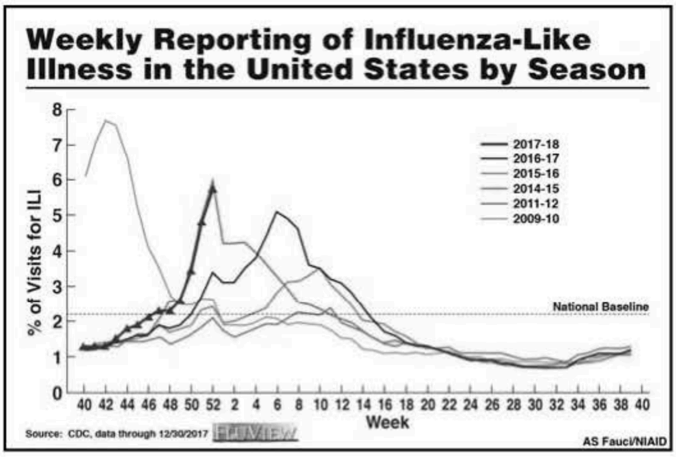

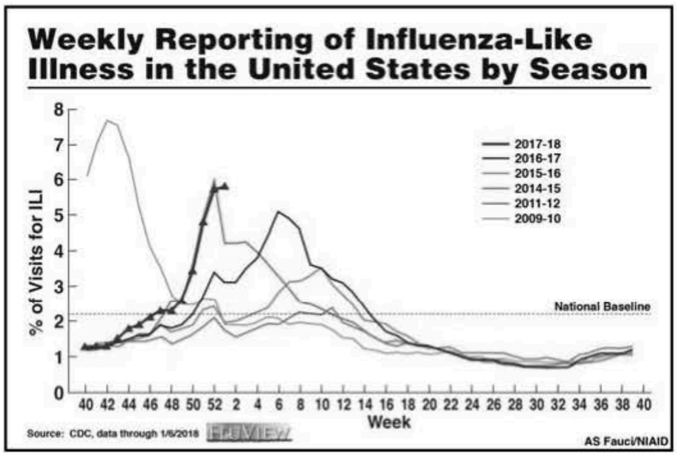

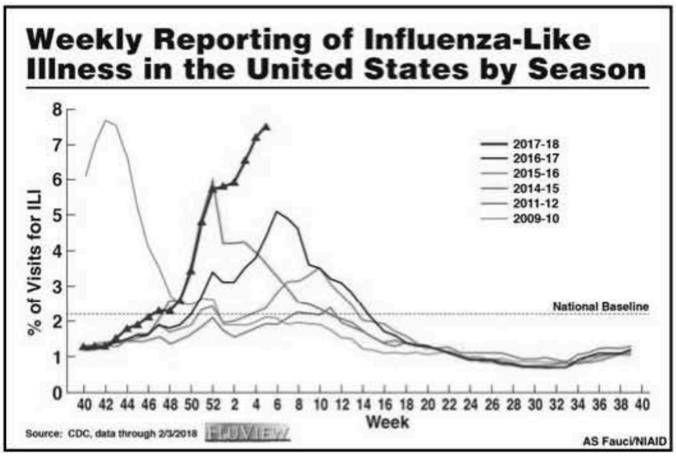

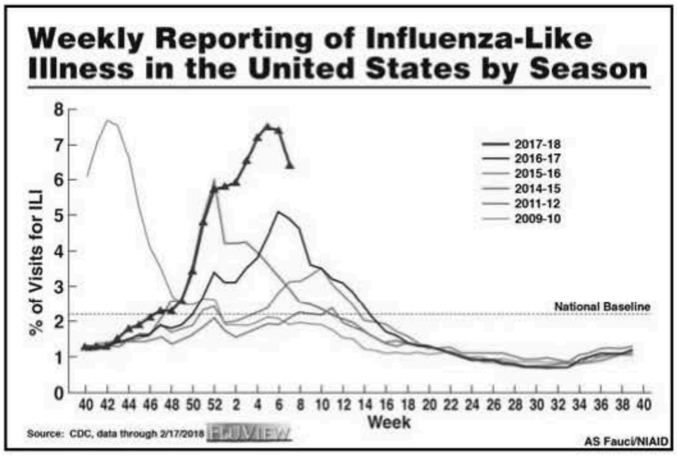

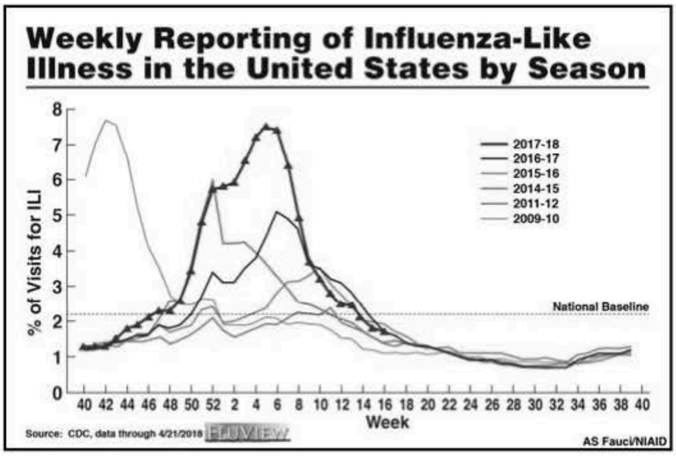

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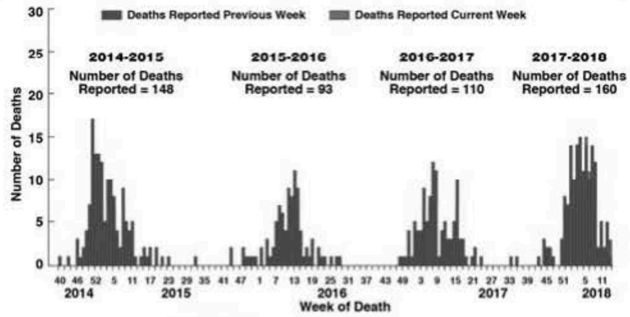
Number of Influenza-Associated Pediatric Deaths by Week of Death



Source: CDC, data through 4/21/2018

AS Fauci/NIAID

Adjusted Influenza Vaccine Effectiveness Estimates, United States

*Interim estimate

AS Fauci/NIAID

Source: CDC

MMWR Vol. 67 No. 6 February 16, 2018

Interim Estimates of 2017-18 Seasonal Influenza Vaccine Effectiveness — United States, February 2018

- Adjusted vaccine effectiveness was 36%
  - 25% against influenza A H3N2 viruses
  - 67% against influenza A H1N1 viruses
  - 42% against influenza B viruses

AS Fauci/NIAID

MMWR Vol. 67 No. 6 February 16, 2018

Interim Estimates of 2017-18 Seasonal Influenza Vaccine Effectiveness — United States, February 2018

- Adjusted vaccine effectiveness was 36%
  - 25% against influenza A H3N2 viruses
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  - 42% against influenza B viruses

AS Fauci/NIAID

STAT

March 9, 2018

Flu Vaccine Grown Without Eggs Provided Measurably Better Protection This Season, FDA Says

AS Fauci/NIAID

Is there more to the story?

AS Fauci/NIAID

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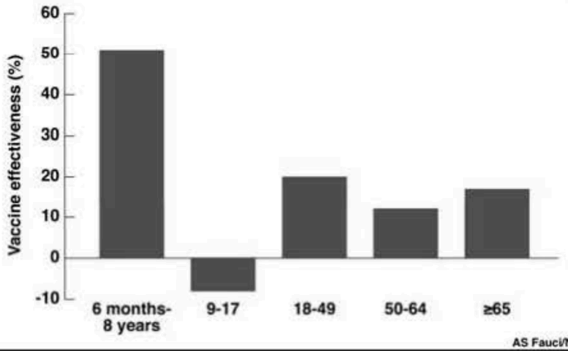
Vaccine Effectiveness Against H3N2 Varies by Age Group



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On the Doctrine of Original Antigenic Sin

Thomas Francis Jr., M.D.
Henry Sweall University Professor Epidemiology, Chairman of the Department of Epidemiology, School of Public Health, and Professor of Epidemiology, Department of Pediatrics and Communicable Diseases, Medical School, University of Michigan

Read April 21, 1960

The antibody of childhood is largely a response to the dominant antigen of the virus causing the first Type A influenza infection of the lifetime. The striking feature is that the antibody which is first established continues to characterize that cohort of the population throughout its life.

PNAS Vol 104 No 6 1960

AS Fauci/NIAID

- Current seasonal influenza vaccines are not consistently effective
- Pandemics do occur and response after the fact is not effective
- "Chasing after" potential pandemic outbreaks (pre-pandemic viruses) is costly and ineffective

AS Fauci/NIAID

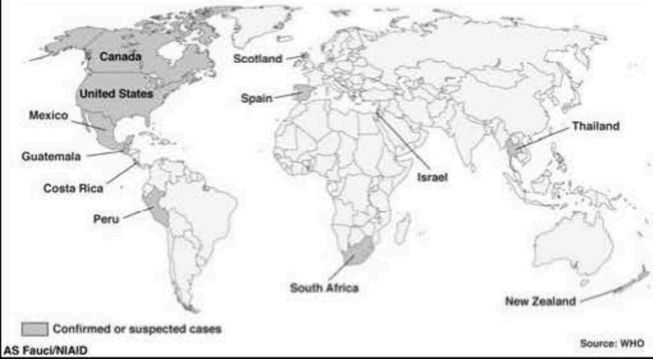
AS Fauci/NIAID

Beginning of Global H1N1 Influenza as of March, 2009

AS Fauci/NIAID

Source: WHO

Global H1N1 Influenza Cases, as of April 29, 2009



AS Fauci/NIAID

Source: WHO

AMERICAN OVERSIGHT

Senate Appropriations Subcommittee Hearing on H1N1 Influenza, April 28, 2009

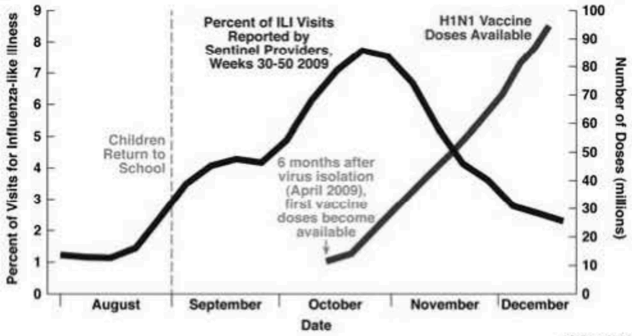
AS Fauci/NIAID

Countries with Confirmed Pandemic (H1N1) 2009 Influenza Cases as of Sept. 30, 2009

AS Fauci/NIAID

Source: WHO

Vaccine Lags Behind 2009 H1N1 Influenza Pandemic



AS Fauci/NIAID

House Oversight and Government Reform Committee Hearing on H1N1 Influenza Vaccines, Sept. 29, 2009

AS Fauci/NIAID

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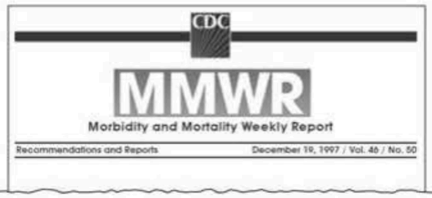
AS Fauci/NIAID



Isolation of Avian Influenza A(H5N1) Viruses from Humans--Hong Kong, May-December 1997

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AMERICAN OVERSIGHT

AS Fauci/NIAID

H5N1 Influenza Cases, 2003-2005

Source: WHO 2/2005

AS Fauci/NIAID

HHS News

U.S. Department of Health and Human Services
www.hhs.gov/news

FOR IMMEDIATE RELEASE
Tuesday, September 21, 2004

HHS Orders Avian Flu Vaccine Doses as Preventive Measure

HHS Secretary Tommy G. Thompson announced today the awarding of a contract to Aventis Pasteur Inc. to manufacture and store 2 million doses of avian influenza H5N1 vaccine, an important initial acquisition to better prepare the nation for an influenza pandemic.

The New York Times

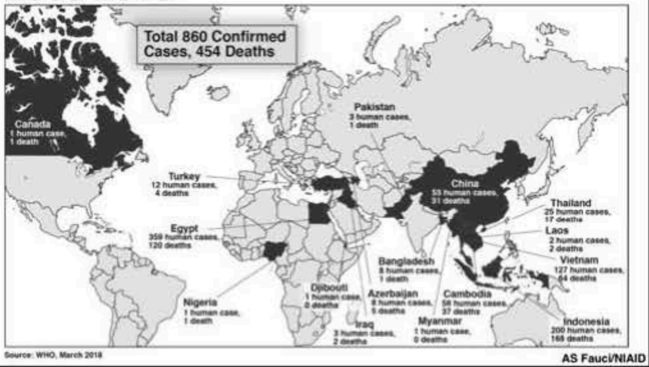
November 1, 2005

Bush Calls for $7.1 Billion to Prepare for Bird Flu Threat

President Bush today unveiled a strategy to combat the threat of an avian flu pandemic, calling for $7.1 billion in emergency spending to stockpile reserves of medicines and to press ahead with the development of a new vaccine.

AS Fauci/NIAID

H5N1 Influenza Cases in Humans, 2003-2018



Source: WHO, March 2018

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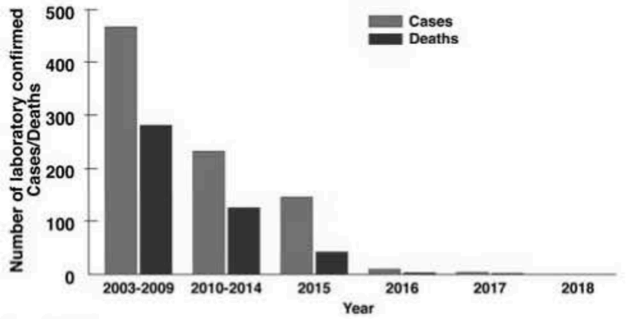
H5N1 Influenza Cases in Humans, 2003-2018



Source: WHO 2018

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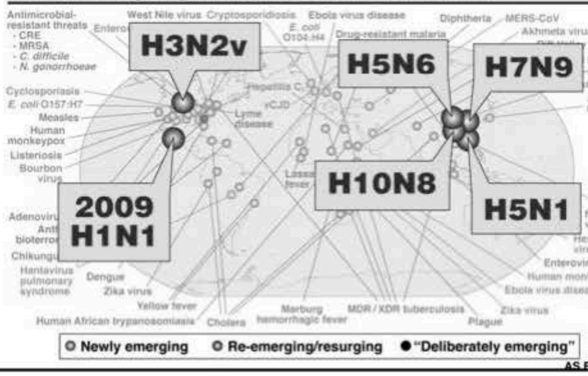
How do we decide which animal viruses to "chase after"?

AS Fauci/NIAID

AMERICAN OVERSIGHT

Global Examples of Emerging and Re-Emerging Infectious Diseases



AS Fauci/NIAID

Published online August 2, 2014
Current Topics in Microbiology and Immunology

Influenza Pathogenesis and Control – Volume I

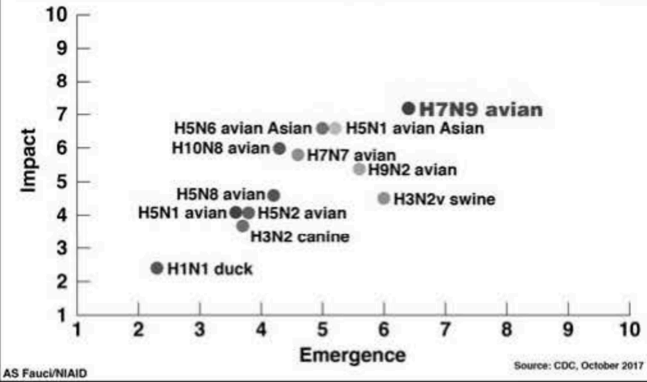
Pandemic Preparedness and the Influenza Risk Assessment Tool (IRAT)

NJ Cox, SC Trock, SA Burke

- Genomic Variation
- Receptor Binding
- Transmission in Laboratory Animals
- Antivirals and Treatment Options
- Existing Population Immunity
- Disease Severity and Pathogenesis
- Antigenic Relationship to Vaccine Candidates
- Global Geographic Distribution
- Infection in Animals, Human Risk of Infection
- Human Infections and Transmission

AS Fauci/NIAID

H7N9: Risk Assessment Using IRAT

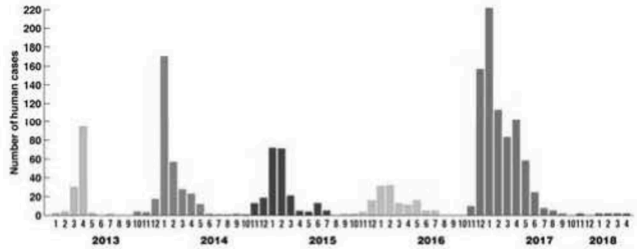


Source: CDC, October 2017

AS Fauci/NIAID

Waves of Human H7N9 Influenza Infections in China, February 2013-present

- **1,625** confirmed human cases, **623** deaths
- 5th wave: >50% of cumulative cases



Source: FAO, 4/25/2018

AS Fauci/NIAID

STAT

May 1, 2017

As Bird Flu Spreads, US Concludes Its Vaccine Doesn't Provide Adequate Protection

AS Fauci/NIAID

NIH National Institutes of Health
Turning Discovery Into Health

FOR IMMEDIATE RELEASE
Thursday, March 15, 2018

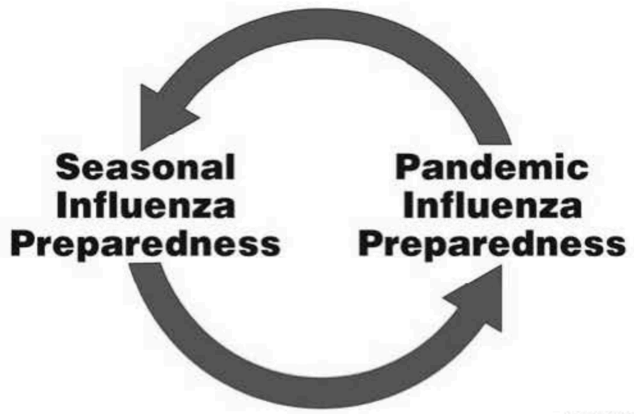
News Release

H7N9 Influenza Vaccine Clinical Trials Begin

- Inactivated 2017 H7N9 influenza vaccine manufactured by Sanofi Pasteur with or without AS03 adjuvant
- Phase 2 trials will enroll up to 420 volunteers ages 19 to 64 years and up to 300 volunteers >65 years of age

AS Fauci/NIAID

AMERICAN OVERSIGHT



AS Fauci/NIAID

Major Challenges in Influenza Vaccinology

- Production of vaccines – Egg-based versus Cell-based versus Recombinant DNA Technology
- Strain-Specificity versus Universal Strain Coverage

AS Fauci/NIAID

Major Challenges in Influenza Vaccinology

- Production of vaccines – Egg-based versus Cell-based versus Recombinant DNA Technology
- Strain-Specificity versus Universal Strain Coverage

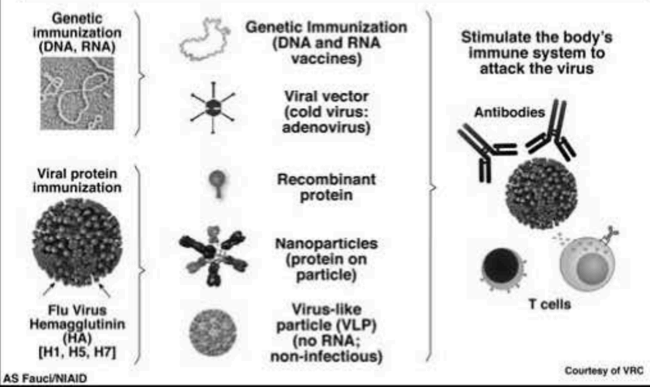
AS Fauci/NIAID

Evolution of Technologies for Influenza Vaccines

Egg-based → Cell-based → Recombinant DNA Technologies

AS Fauci/NIAID

Vaccine Platform Technologies



AS Fauci/NIAID

Courtesy of VRC

Major Challenges in Influenza Vaccinology

- Production of vaccines – Egg-based versus Cell-based versus Recombinant DNA Technology
- Strain-Specificity versus Universal Strain Coverage

AS Fauci/NIAID

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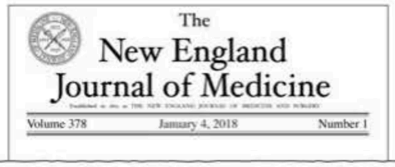
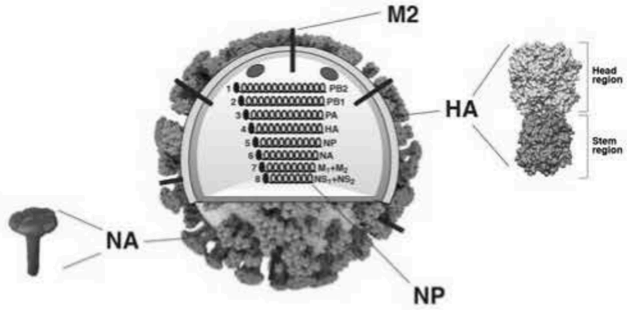


Chasing Seasonal Influenza — The Need for a Universal Influenza Vaccine

CI Paules, SG Sullivan, K Subbarao, and AS Fauci

AS Fauci/NIAID

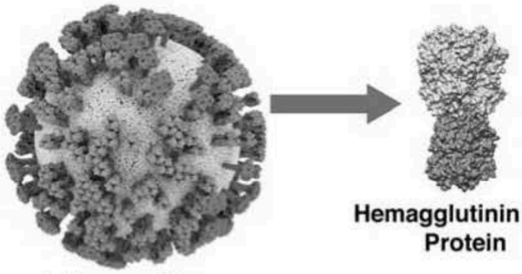
Selected Targets for "Universal" Influenza Vaccines



AS Fauci/NIAID

Courtesy of VRC

Hemagglutinin Protein: Major Target of Influenza Vaccines



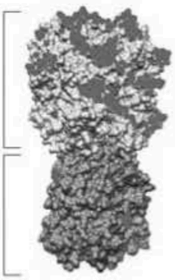
Influenza Virus

Hemagglutinin (HA) Protein

AS Fauci/NIAID

Courtesy of VRC

Influenza A Hemagglutinin (HA)



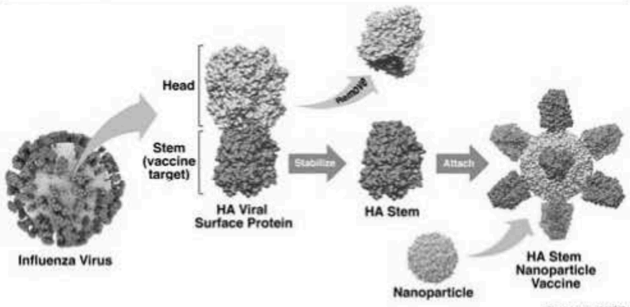
Head region
- Target of current influenza vaccines
- Differs among influenza strains
- Many mutations (●) each season

Stem region
- Target of universal influenza vaccines
- Similar among influenza strains
- Few mutations each season

AS Fauci/NIAID

Courtesy of VRC

Representative Approach to the Development of a Universal Influenza Vaccine



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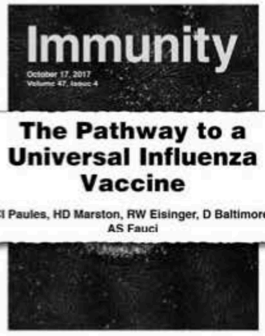

The Pathway to a Universal Influenza Vaccine

CI Paules, HD Marston, RW Eisinger, D Baltimore, AS Fauci

AS Fauci/NIAID

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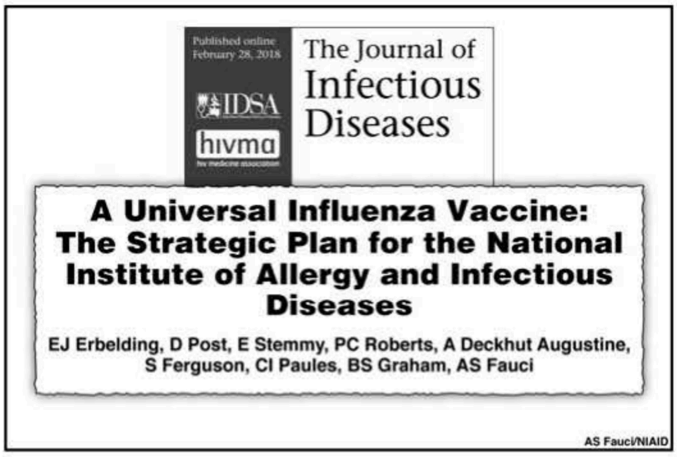

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From: Eisinger, Robert (NIH/NIAID) [E]
Sent: Fri, 11 May 2018 11:15:47 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: RE: Pre-submission draft of Lancet Commission on TB

I am reviewing the document and asked for an appt. late today for Laurie and I to discuss our comments with you.
Thanks
Bob

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Friday, May 11, 2018 7:14 AM
To: Eisinger, Robert (NIH/NIAID) [E] (b)(6)
Subject: FW: Pre-submission draft of Lancet Commission on TB

Let us discuss.

From: McKey, David (b)(6) **On Behalf Of** Goosby, Eric
Sent: Thursday, May 10, 2018 5:14 PM
To: Goosby, Eric (b)(6)
Cc: Reid, Michael (b)(6) McKey, David (b)(6) Soumya Swaminathan (b)(6) Jamison, Dean (b)(6)
Subject: Pre-submission draft of Lancet Commission on TB

Dear Commissioners,

Thanks to all of you for sending edits and comments on the draft of the Commission report that Mike sent around 2 weeks ago. We have received many constructive edits/emails/phone calls. Further, I think the revised version, attached , has been substantially improved based on that feedback.

Acknowledging that the current draft is still incomplete (some small pieces of analyses are still awaited) and that we can't please everyone (smile), but mindful of the fact that we must submit now if we want to ensure that publishing at the time of the HLM remains possible, Mike will plan on submitting this draft to The Lancet this Sunday evening, **May 13**.

While we welcome feedback before then, I am sure that there will be more opportunities for edits and improvement once the draft has been reviewed by the external reviewers and The Lancet editorial team. I am also very open to arranging

AMERICAN OVERSIGHT

a conference call to discuss any parts of the report that Commissioners want to discuss in more depth.

I am attaching a revised draft of Executive Summary and main report manuscript with this email. Additional panels/figures/tables are also available to review at the links below (as are individual sections of the report). In addition to the full manuscript, I am also attaching the front page with Commissioners and co-authors listed. Kindly review the affiliations associated with your name and email David McKey, ccing Mike, if these need to be amended.

Box Users:
Report Sections
Tables/Figures
Panels/Appendices
Track change version of Report Sections

Dropbox Users:
Report Sections
Tables/Figures
Panels/Appendices
Track change version of Report Sections

Finally, Mike or David will be contact shortly to ask you complete a Conflict of Interests form that is a requirement of The Lancet submission process. Mike will also be reaching out to non-commissioner co-authors listed on the title page, to ensure they have a chance to review the draft before submission. If there are contributing authors that worked on the report not included on the Title page, please let Mike know as soon as possible.

Thanks again for your partnership in this effort.

Best regards

Eric

Eric P. Goosby, M.D.
Professor of Medicine
UN Secretary-General's Special Envoy on TB
Director, Global Health Delivery & Diplomacy
Institute of Global Health Sciences
University of California, San Francisco

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Fri, 11 May 2018 11:13:53 +0000
To: Eisinger, Robert (NIH/NIAID) [E]
Subject: FW: Pre-submission draft of Lancet Commission on TB
Attachments: Main Manuscript_051018.docx, Title Page_051018.docx, TB Executive summary_051018.docx

Let us discuss.

From: McKey, David (b)(6) **On Behalf Of** Goosby, Eric
Sent: Thursday, May 10, 2018 5:14 PM
To: Goosby, Eric <Eric.Goosby@ucsf.edu>
Cc: Reid, Michael <Michael.Reid2@ucsf.edu>; McKey, David (b)(6) Soumya Swaminathan (b)(6) Jamison, Dean (b)(6)
Subject: Pre-submission draft of Lancet Commission on TB

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Thanks again for your partnership in this effort.

Best regards

Eric

Eric P. Goosby, M.D.
Professor of Medicine
UN Secretary-General's Special Envoy on TB
Director, Global Health Delivery & Diplomacy
Institute of Global Health Sciences
University of California, San Francisco

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Fri, 11 May 2018 11:11:54 +0000
To: NIAID OD AM
Subject: FW: Invitation to Speak at Global Health & Innovation Conference at Yale in April

Did we already discuss this? If not, let us decide.

From: Jennifer Staple-Clark (b)(6)
Sent: Thursday, May 10, 2018 3:48 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: Invitation to Speak at Global Health & Innovation Conference at Yale in April

Dear Anthony,

I hope that this email finds you well. We are delighted to invite you to speak at the 16th annual Global Health & Innovation Conference at Yale on April 13-14, 2019. Presented annually by Unite For Sight, the Global Health & Innovation Conference is the world's largest global health conference as well as the world's largest social entrepreneurship conference. The conference annually convenes 2,000 leaders, changemakers, and participants from all fields of global health, international development, and social entrepreneurship. The Global Health & Innovation Conference is working to advance dialogue and conversation about health, development, education, and entrepreneurship outcomes, and the audience would benefit tremendously from hearing your perspectives and expertise.

For your reference, you may see the past 2018 conference schedule as well as a 4-minute video about the conference. Past conference speakers have ranged from Jeffrey Sachs and Jacqueline Novogratz to Nicholas Kristof, Leana Wen, Seth Godin, and Al Sommer.

Thank you for your consideration, and for all that you do. I look forward to hearing from you and very much hope that you will be available to speak at the 16th annual Global Health & Innovation Conference on April 13-14, 2019.

Best regards,
Jennifer Staple-Clark
Founder and Chief Executive Officer
Unite For Sight

AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Fri, 11 May 2018 11:10:00 +0000
To: NIAID OD AM
Subject: FW: Invitation to Morehouse School of Medicine - New Office of Global Health Equity Kick-off!

Let us discuss. This is a tough one since this is a Minority Institution.

From: Bastien, Gilberte (b)(6)
Sent: Thursday, May 10, 2018 2:59 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: Invitation to Morehouse School of Medicine - New Office of Global Health Equity Kick-off!

Good afternoon Dr. Fauci,

It was great to see you at the recent Fogarty at 50 symposium and to chat briefly about the launching of our new Office of Global Health Equity here at Morehouse School of Medicine.

As we prepare to introduce the office to Atlanta's broader global health community, our team was united in the belief that NIAID should be represented in this unveiling event. We would love to have you join us and to provide remarks around NIAIDs global health related priorities.

We are hoping to hold this kick-off, day-long meeting in the early fall (September - October). Please let us know if you would be interested in attending and speaking at this important milestone for our evolving global health work at Morehouse, and let us know a few dates that would work best for you during our anticipated time frame. We are committed to being as flexible as possible on our end to ensure you can participate in this important meeting.

Per your suggestion, I have copied Ms. Conrad for help with scheduling and identification of possible dates for the upcoming fall. We look forward to hearing from you and hopefully to hosting you in the fall!

Best regards,
Gigi

Gilberte ("Gigi") Bastien, Ph.D.
Assistant Director - Office of Global Health Equity
Assistant Professor - Department of Psychiatry and Behavioral Sciences
Morehouse School of Medicine
720 Westview Drive, SW
Atlanta, GA 30310

AMERICAN OVERSIGHT

Office: (b)(6)
E-mail: (b)(6)

"You cannot fully understand your own life without knowing and thinking beyond your life, your own neighborhood, and even your own nation." -- Dr. Johnetta Betsch Cole

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AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Fri, 11 May 2018 10:08:07 +0000
To: Auchincloss, Hugh (NIH/NIAID) [E]
Cc: Conrad, Patricia (NIH/NIAID) [E]
Subject: FW: The Vaccine Education Project

Let us discuss.

From: Edward Lewin (b)(6)
Sent: Thursday, May 10, 2018 10:51 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Walt Orenstein (b)(6)
Subject: Re: The Vaccine Education Project

Dear Tony,

It was wonderful seeing you at the NFID event. I thought your comments were heartfelt and right on. I'm glad I wax in the room to hear them....

I want to ask you to consider partnering with Walt, me and our distinguished Board in engaging Middle School kids in enlisting their support and
that of their families and teachers. We're hoping to get started soon (in time for the Fall, 2018 semester) and would love to partner with yo in so doing.
Please let me know if you would like me to sto! by to discuss.

Best regards,
Ed.

On Sat, Apr 8, 2017 at 7:17 AM, Fauci, Anthony (NIH/NIAID) [E] (b)(6) wrote:
Ed:
Thanks for the note. I hope that all is well with you.
Best regards,
Tony

From: Edward Lewin (b)(6)
Sent: Saturday, April 08, 2017 12:20 AM
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Orenstein, Walter; Offit, Paul; Hotez, Peter Jay; John Modlin; Sam Katz; Schaffner, William; Stanley Plotkin
Subject: The Vaccine Education Project

Dear Tony,

I saw the CNN program tonight and the discussion afterward. I thought both were terrific and very timely.

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One of the themes of the evening centered on vaccine hesitancy which, in some quarters of the country and population, is (as you know better than anyone) increasing.

In June, 2015, I sent you a precis of the VEP. We are still looking for funding to execute this study which, we think, is a unique and potent approach to this important problem. It's an attempt to advance vaccine acceptance by looking to the upcoming generation.

We are applying to the NIH (SEPA, Tony Beck's program), RWJF and the Pichichero Family Research Foundation. If you have any other suggestions, please let me know.

Thank you for all you do for all of us....

Best regards,
Ed. (b)(6)

We believe in the ability of children to understand concepts without prejudice if they are presented in an honest, straightforward, accurate and palatable way. On the recommendation of our education colleagues, we chose to begin with middle-schoolers because 1) they are capable of understanding scientific concepts, 2) at their age, they are receiving a number of vaccines (e.g., influenza, Tdap, meningococcus and HPV) that make the material relevant, and 3) for the most part, they have not been subject to prejudicial misinformation, nor are they jaded.

We have developed a two-phase program. Phase I, supported by the CDC, saw the development of a curriculum for these kids in vaccine science presented in an entertaining and informative format of 13 videos. This has been completed.

Phase II is upcoming and will: 1) Provide the curriculum in the form of these videos which are intended to be viewed at home in order to to avoid taking time from class activities. Classroom time is limited to discussions of the material viewed at home. 2) The curriculum will then undergo extensive focus groups to assure student acceptance. 3) This will be followed by a controlled study designed to test the efficacy of changing the student's knowledge of vaccines as a result of having viewed the curriculum. The focus groups and experimental phase have been structured to assure attention to educational demographics, economic background and school performance. 4) Following these activities, the curriculum will be made available nationally, beginning with those school districts with a >5% philosophical exemption rate.

We believe that presenting this curriculum to kids on vaccine science will serve not only to educate them on this subject, but will serve as an object lesson on how to approach any matter with scientific content.

I have enclosed an example of the videos we produced.

We believe that this project is an excellent candidate for the RA-21 program.

http://www.youtube.com/watch?v=0dvZZSif6rg

--
Edward B. Lewin, M.D., FAAP, PIDS, IDSA

"There is nothing like a dream to create the future"

AMERICAN OVERSIGHT

--
Edward B. Lewin, M.D., FAAP, PIDS, IDSA

"There is nothing like a dream to create the future"

AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Thu, 10 May 2018 21:19:38 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Meeting with Dr. Poonawalla and Mr. Poonawalla (father and son) founder and CEO of the Serum Institute of India
Attachments: RE: Meeting Request - Serum Institute Leadership, Briefing documents for Serum Inst Visit June 5, 2018; Pre-brief, May 31, 2018, India 5_30_2018.docx

Greg, I think a 10 minute overview presentation would be a great way to start the meeting.

He did similar when he met with the CEO/son, Mr. Poonawalla last year. The meeting went very well; they hit it off. With the founder/father, Dr. Poonawalla, at this meeting, making a similar start would be helpful to kick of a general discussion.

He might especially focus on intramural (VRC and DIR) efforts to discover and develop vaccines and then link to industry to carry them forward. Serum has licensed Whitehead live attenuated dengue vaccine and will undertake phase III trials probably in 2019. They are interested in VRC vaccines as well.

Let me know if you need anything. G

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Thu, 10 May 2018 21:18:45 +0000
To: Handley, Gray (NIH/NIAID) [E]
Cc: Auchincloss, Hugh (NIH/NIAID) [E];Vance, Meaghan (NIH/NIAID) [C];Greg Folkers (b)(6)
Subject: RE: Meeting Request - Serum Institute Leadership

Gray – ASF said he would do this for 30 mins. Am holding 12 pm – 12:30 pm on his calendar. Please advise as to what if any presentation ASF would need.

Also – will you want a pre-brief and when?

thx

Patricia L. Conrad
Special Assistant to the Director

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From: Handley, Gray (NIH/NIAID) [E]
Sent: Tuesday, May 08, 2018 6:25 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Auchincloss, Hugh (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Subject: Meeting Request - Serum Institute Leadership

Would ASF have any time for a 30-60 minute meeting with Dr. Poonawalla and Mr. Poonawalla (father and son) on June 5 any time after 11 a.m.? The Poonawallas are the founder and CEO of the Serum Institute of India, which makes about 60% of the world's vaccines.

They have asked for a courtesy meeting to discuss their interest in working with NIAID and vaccine trials underway. They also own the Serum Institute – which is entirely privately owned.

ASF has previously met Mr. Poonawalla and they hit it off.

Thanks. Gray

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From: Handley, Gray (NIH/NIAID) [E]
Sent: Wed, 30 May 2018 21:45:00 +0000
To: Vance, Meaghan (NIH/NIAID) [C];Conrad, Patricia (NIH/NIAID) [E]
Cc: Auchincloss, Hugh (NIH/NIAID) [E];Hudgings, Carole (NIH/NIAID) [E];Gupta, Ranjan (NIH/NIAID) [E];Meegan, James (NIH/NIAID) [E]
Subject: Briefing documents for Serum Inst Visit June 5, 2018; Pre-brief, May 31, 2018
Attachments: Visitor Bios -SIIPL June 5 2018.docx, SIIPL Profile and NIH-NIAID Engagements - June 2018.docx, India 5_30_2018.docx, ASF-Briefing Memo India-Poonawalla-SII-DRAFT5 6-5-18fgh.doc
Importance: High

Here are materials for tomorrow's pre-brief and the meeting next week with Serum Institute leadership.

Please print and provide to Dr. Fauci and any other participants.

See you tomorrow.

Gray

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AMERICAN OVERSIGHT

From: Conrad, Patricia (NIH/NIAID) [E] on behalf of Fauci, Anthony (NIH/NIAID) [E]
Sent: Thu, 10 May 2018 21:14:04 +0000
To: NIAID OD AM
Subject: FW: Invitation to Speak at Global Health & Innovation Conference at Yale in April

Patricia L. Conrad
Special Assistant to the Director

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From: Jennifer Staple-Clark (b)(6)
Sent: Thursday, May 10, 2018 3:48 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: Invitation to Speak at Global Health & Innovation Conference at Yale in April

Dear Anthony,

I hope that this email finds you well. We are delighted to invite you to speak at the 16th annual Global Health & Innovation Conference at Yale on April 13-14, 2019. Presented annually by Unite For Sight, the Global Health & Innovation Conference is the world's largest global health conference as well as the world's largest social entrepreneurship conference. The conference annually convenes 2,000 leaders, changemakers, and participants from all fields of global health, international development, and social entrepreneurship. The Global Health & Innovation Conference is working to advance dialogue and conversation about health, development, education, and entrepreneurship outcomes, and the audience would benefit tremendously from hearing your perspectives and expertise.

For your reference, you may see the past 2018 conference schedule as well as a 4-minute video about the conference. Past conference speakers have ranged from Jeffrey Sachs and Jacqueline Novogratz to Nicholas Kristof, Leana Wen, Seth Godin, and Al Sommer.

Thank you for your consideration, and for all that you do. I look forward to hearing from you and very much hope that you will be available to speak at the 16th annual Global Health & Innovation Conference on April 13-14, 2019.

Best regards,
Jennifer Staple-Clark
Founder and Chief Executive Officer
Unite For Sight

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From: Kralievits, Katherine
Sent: Thu, 10 May 2018 19:52:55 +0000
To: mrd54;Sullivan, Kevin;Barbara Bush;Fauci, Anthony (NIH/NIAID)
(b)(6) (b)(6)
(b)(6) Farmer, Paul; (b)(6) Yvette Umwali
Cc: Prince, Emma;Walker, Annie
Subject: Re: The Lazarus Effect: 15 Years Later Video

Dear Sully,

Thank you so much to you, Emma, Annie, and your team for hosting an incredible event. It was both inspirational and informative, and we're so grateful for your hard work and attention to every detail.

Paul was especially grateful for the opportunity to reunite with his close friends and to celebrate this initiative and President Bush.

Again, thank you, and best wishes,
Katie

--
Katherine Kralievits, MS
Office of Dr. Paul Farmer
Department of Global Health and Social Medicine
Harvard Medical School | Partners In Health
(b)(6)

From: mrd54 (b)(6)
Date: Thursday, May 10, 2018 at 12:55 PM
To: "Sullivan, Kevin" (b)(6) Barbara Bush (b)(6)
(b)(6) (b)(6) (b)(6)
(b)(6) (b)(6) (b)(6)
(b)(6) (b)(6)
(b)(6) (b)(6) "Farmer, Paul"
(b)(6) (b)(6)
(b)(6) Yvette Umwali (b)(6) "Kralievits, Katherine" (b)(6)
Cc: "Prince, Emma" (b)(6) "Walker, Annie" (b)(6)
Subject: Re: The Lazarus Effect: 15 Years Later Video

Thanks sully to you and the team for all the hard work.

It was a great and humbling privilege to be part of the event and to be among such an extraordinary group.

Take care

AMERICAN OVERSIGHT

Mark

Sent via the Samsung Galaxy S7, an AT&T 4G LTE smartphone

-------- Original message --------
From: "Sullivan, Kevin" (b)(6)
Date: 5/10/18 11:47 AM (GMT-06:00)
To: Barbara Bush (b)(6) (b)(6) (b)(6)
(b)(6) (b)(6) alice (b)(6)
(b)(6) (b)(6) Mark Dybul
(b)(6) Yvette Umwali (b)(6)
(b)(6)
Cc: "Prince, Emma" (b)(6) "Walker, Annie" (b)(6)
Subject: The Lazarus Effect: 15 Years Later Video

Good morning all,

On behalf of Emma, Annie and our Events team, thank you so much for all you did to make last night such a memorable event. We have gotten terrific feedback - there is no question our audience was inspired about what's been accomplished...and understands the importance of keeping PEPFAR funded and strong.

Here is a link to the video from bushcenter.org. Feel free to share freely:

http://www.bushcenter.org/exhibits-and-events/events/2018/05/engage-lazarus-effect.html

Please don't hesitate to reach out if I can be helpful on this end.

Sincerely,

Sully

Kevin Sullivan
Senior Advisor, External Affairs
George W. Bush Presidential Center

T (b)(6) F 214.855.4347 M (b)(6)
(b)(6)

AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Wed, 9 May 2018 17:44:13 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Call with Senator Markey - univ flu vax budget
Attachments: RE: Update: Flu work briefing

AMERICAN OVERSIGHT

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Wed, 9 May 2018 17:42:44 +0000
To: (b)(6) Haskins, Melinda (NIH/NIAID) [E]
Cc: (b)(6) Vance, Meaghan (NIH/NIAID) [C]
Subject: RE: Update: Flu work briefing

3 pm ET tomorrow will work. We will call the number provided. The number for our office in case you need to call us is (b)(6)

Thank you,
-patty

Patricia L. Conrad
Special Assistant to the Director

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From: (b)(6)
Sent: Wednesday, May 09, 2018 1:31 PM
To: (b)(6) Haskins, Melinda (NIH/NIAID) [E]
(b)(6)
Cc: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
(b)(6)
Subject: RE: Update: Flu work briefing

(b)(6)

Would a call at 3pm tomorrow work on your end?

Dr. Fauci can call my direct extension at (b)(6) and I can connect him to the Senator from there.

Please advise- thanks!

(b)(6)

From: (b)(6)
Sent: Wednesday, May 9, 2018 1:01 PM
To: Haskins, Melinda (NIH/NIAID) [E] (b)(6)

AMERICAN OVERSIGHT

Cc: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
(b)(6)
Subject: RE: Update: Flu work briefing

Thanks, Melinda! Looping in folks from our team to see what the best option may be. Appreciate your speedy response!

From: Haskins, Melinda (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, May 9, 2018 12:53 PM
To: (b)(6)
Cc: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: RE: Update: Flu work briefing

(b)(6)

Dr. Fauci is on travel today to attend an event. He would be available to speak to the Senator before 3 p.m., today; or tomorrow afternoon. Please let me know how you would like to proceed and at what number you would like Dr. Fauci to call the Senator.

Thanks,
Melinda

From: (b)(6)
Sent: Wednesday, May 9, 2018 11:30 AM
To: Haskins, Melinda (NIH/NIAID) [E] (b)(6)
Cc: (b)(6)
Subject: RE: Update: Flu work briefing

Thanks!

From: Haskins, Melinda (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, May 9, 2018 11:02 AM
To: (b)(6)
Subject: RE: Update: Flu work briefing

(b)(6)

Thanks for reaching out. We will be in touch shortly.

Melinda

From: (b)(6)
Sent: Wednesday, May 9, 2018 10:56 AM
To: Haskins, Melinda (NIH/NIAID) [E] (b)(6)
Cc: (b)(6)
Subject: RE: Update: Flu work briefing

AMERICAN OVERSIGHT

Hi Melinda – hope you're doing well!

I'm reaching out because Sen. Markey would like to call Dr. Fauci to discuss recent developments regarding the universal flu vaccine, specifically the appropriations increase for FY18. I've copied my colleague, Sarah, who would be able to help coordinate a time on the Senator's end. Would you be able to help us arrange that, please?

Thank you so much!
(b)(6)

From: Haskins, Melinda (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, January 24, 2018 7:15 PM
To: (b)(6)
Subject: Re: Update: Flu work briefing

Thanks

Sent from my iPhone

On Jan 24, 2018, at 3:42 PM, (b)(6) wrote:

Running behind – should be reaching out shortly!

From: (b)(6)
Sent: Wednesday, January 24, 2018 10:24 AM
To: 'Haskins, Melinda (NIH/NIAID) [E]' (b)(6)
Cc: (b)(6) Klimczak, Kate (NIH/OD) [E] (b)(6) Berkson, Laura (NIH/OD) [E] (b)(6) Selgrade, Sara (NIH/NIAID) [E] (b)(6)
Subject: RE: Update: Flu work briefing

Thanks so much! Let's aim for 3:30 and will try to shoot you an email if something changes with the timing.

Thank you again for your speedy work on this – we really appreciate it!

From: Haskins, Melinda (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, January 24, 2018 10:11 AM
To: (b)(6)
Cc (b)(6) Klimczak, Kate (NIH/OD) [E] (b)(6) Berkson, Laura (NIH/OD) [E] (b)(6) Selgrade, Sara (NIH/NIAID) [E] (b)(6)
Subject: Update: Flu work briefing

(b)(6)

AMERICAN OVERSIGHT

Dr. Fauci would be available to speak with Senator Markey after 3 p.m., today. Dr. Fauci can be reached at (b)(6) If this time is inconvenient for the Senator, we will work something out.

Sincerely,

Melinda Haskins
Chief, NIAID OCGR LACMB
Direct: (b)(6)

From: Berkson, Laura (NIH/OD) [E]
Sent: Wednesday, January 24, 2018 9:31 AM
To: (b)(6) Haskins, Melinda (NIH/NIAID) [E]
(b)(6)
Cc: (b)(6) Klimczak, Kate (NIH/OD) [E]
(b)(6)
Subject: RE: Potential flu work briefing?

Hi (b)(6)

No problem – happy to help! I'm looping in my colleague, Melinda Haskins, who works at NIAID and can help set something up.

Best,
Laura

From: (b)(6)
Sent: Tuesday, January 23, 2018 6:45 PM
To: Berkson, Laura (NIH/OD) [E] (b)(6)
Cc: (b)(6)
Subject: RE: Potential flu work briefing?

Hi Laura – so sorry about this, but it looks like Sen. (b)(6) would like to call Dr. Fauci directly to ask some of these questions. Do you have his contact information?

I think he's particularly interested in Dr. Fauci's estimates on how much we'd need to invest to create a universal flu vaccine and develop new/updated models for predicting the flu and producing the vaccine. Happy to hop on the phone tomorrow morning if that would be helpful.

Thanks, and again, apologies for the last minute request. Thanks, Laura!

From: (b)(6)
Sent: Tuesday, January 23, 2018 6:22 PM
To: 'Berkson, Laura (NIH/OD) [E]' (b)(6)
Cc: (b)(6)
Subject: Potential flu work briefing?

Hi Laura – thanks again for your help with the letter last week!

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Following-up on that, do you think it would be possible for us to schedule a call with the relevant folks at NIAID to discuss some of the flu-related work at the agency? We're specifically interested in updates on Dr. Fauci's work to create a universal flu vaccine, and how much the agency thinks it would cost to develop it; what kind of big data or algorithmic work the NIAID is doing, probably in conjunction with other agencies, to better predict the strain in the upcoming flu season; and the agency's work around cell-based flu vaccines.

Do you think this is something we'd be able to schedule for later this week? Please let me know if I can provide any additional information.

Thanks so much!

(b)(6)

AMERICAN OVERSIGHT

From: Billet, Courtney (NIH/NIAID) [E]
Sent: Wed, 9 May 2018 18:06:19 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Folkers, Greg (NIH/NIAID) [E];Conrad, Patricia (NIH/NIAID) [E];Stover, Kathy (NIH/NIAID) [E]
Subject: ASF: For your review, HVAD Statement
Attachments: HVAD statement 2018 to ASF.docx, NSWB_Clearance_Sheet - HVAD 2018 Statement.doc

Attached for your review is our draft statement for HIV Vaccine Awareness day (May 18). We still don't know the pub date for Peter Kwong's new antibody finding, so that is noted as pending. We've set this up to be co-authored with Maureen Goodenow, at Carl's suggestion. Apparently the co-authorship means quite a lot to her.

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AMERICAN OVERSIGHT

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Wed, 9 May 2018 16:32:29 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: FW: Interview request: Launch of Phase 2 Universal Flu Vaccine Study

As discussed

Reporter: Shelia Poole
Organization: The Atlanta Journal Constitution
Phone: (b)(6)
Email: Shelia.Poole@ajc.com
Subject: Launch of Phase 2 universal influenza vaccine study; role of diversity in clinical trial cohorts

Patricia L. Conrad
Special Assistant to the Director

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From: Deatrick, Elizabeth (NIH/NIAID) [C]
Sent: Wednesday, May 09, 2018 11:16 AM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: NIAID FOG (b)(6) NIAID COGCORE (b)(6) NIAID Media Inquiries (b)(6)
Subject: RE: Interview request: Launch of Phase 2 Universal Flu Vaccine Study

Hi Patty,

This reporter from the Atlanta Journal Constitution just got back to me; she's back in town and would like an interview on just the Phase 2 BiondVax universal flu vaccine trial. She's fine with skipping the diversity in clinical trials questions.

She'd like an interview this afternoon, if possible--Is Dr. Fauci interested/available?

Best,
Elizabeth

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Friday, May 4, 2018 3:30 PM
To: Deatrick, Elizabeth (NIH/NIAID) [C] (b)(6)
Cc: NIAID FOG (b)(6) NIAID COGCORE (b)(6) NIAID Media Inquiries (b)(6)
Subject: RE: Interview request: Launch of Phase 2 Universal Flu Vaccine Study

ASF is happy to do the Phase 2 part but not the other. IF that is ok with them – let us know and we will call the reporter this afternoon.

Let us know as soon as possible. thx

Patricia L. Conrad
Special Assistant to the Director

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From: Deatrick, Elizabeth (NIH/NIAID) [C]
Sent: Friday, May 04, 2018 3:21 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: NIAID FOG (b)(6) NIAID COGCORE (b)(6) NIAID Media Inquiries (b)(6)
Subject: RE: Interview request: Launch of Phase 2 Universal Flu Vaccine Study

Reporter: Shelia Poole
Organization: The Atlanta Journal Constitution
Phone: 404-526-5488
Email: (b)(6)
Subject: Launch of Phase 2 universal influenza vaccine study; role of diversity in clinical trial cohorts
Deadline: next week (reporter is out of town until Tuesday May 8)

Hi Patty,

This reporter is writing two separate articles, and is seeking an expert for both of them. The first is covering the Phase 2 universal flu vaccine study we publicized today (below), and the second is on the importance of including a diversity of subjects in clinical trials.

Would Dr. Fauci be interested in speaking with her for either/both of these? (We can also direct her to Bld. 1 to ask about the diversity issue; I could see her being interested in the All of Us project.)

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FOR IMMEDIATE RELEASE
Friday, May 4, 2018

Media Contact:
Elizabeth Deatrick
(b)(6)
(b)(6)

NIAID-Sponsored Trial of a Universal Influenza Vaccine Begins

A Phase 2 clinical trial of an investigational universal influenza vaccine intended to protect against multiple strains of the virus has begun in the United States. The study is sponsored by the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health, and is being conducted at four U.S. sites that are part of the NIAID-funded Vaccine and Treatment Evaluation Units (VTEUs). The trial is testing an experimental vaccine called M-001 for safety and its ability to produce potentially broad protective immune responses, both on its own and when followed by a standard, licensed seasonal influenza vaccine.

Influenza viruses mutate constantly, resulting in the emergence of viruses that may not always match those targeted by seasonal and pre-pandemic influenza vaccines. Seasonal influenza vaccines are made anew each year to match the strains predicted to circulate in the upcoming season. To receive the best protection against influenza, people must be vaccinated annually. However, if a particular influenza strain changes in an unanticipated way, or a different strain from that included in the vaccine spreads widely, the seasonal influenza vaccine may not be sufficiently protective. Each year, seasonal influenza sickens millions in the United States and results in 140,000 to 710,000 hospitalizations and between 12,000 and 56,000 deaths, according to the Centers for Disease Control and Prevention.

Influenza pandemics occur when a novel influenza strain for which people have little to no protection begins to spread among humans and present a greater public health threat than seasonal influenza. For example, the 1918 influenza pandemic killed at least 50 million people worldwide. An ideal universal influenza vaccine would provide durable protection for all age groups against multiple influenza strains, including those that might cause a pandemic.

“The 2017-2018 influenza season in the United States was among the worst of the last decade and serves as a reminder of the urgent need for a more effective and broadly protective influenza vaccine,” said NIAID Director Anthony S. Fauci, M.D. “An effective universal influenza vaccine would lessen the public health burden of influenza, alleviate suffering and save lives. There are numerous paths of inquiry that the scientific community is pursuing, with each new study yielding more critical information and bringing us closer to our shared goal.”

The new trial is being led by principal investigator Robert L. Atmar, M.D., of Baylor College of Medicine in Houston. The trial will test the M-001 vaccine candidate, developed and produced by BiondVax Pharmaceuticals based in Ness Ziona, Israel. The experimental M-001 vaccine contains antigenic peptide sequences shared among many different influenza viruses. Theoretically, it could protect against many current and emerging strains of influenza. Six previous clinical trials involving a total of 698 participants conducted by BiondVax in Israel and Europe indicated that the vaccine candidate was safe, well-tolerated and produced an immune response to a broad range of influenza strains.

The new study will enroll up to 120 healthy volunteers between the ages of 18 and 49 years. Participants will be assigned randomly to receive either two doses of the experimental vaccine or a

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placebo. They will be vaccinated twice, receiving one dose (1 mg; 0.4 milliliters) of M-001 or placebo via intramuscular injection on the first day and a second dose 22 days later. Approximately 172 days later, all participants will receive an approved seasonal influenza vaccine. During periodic additional clinic visits throughout the course of the study, blood will be drawn from study volunteers to evaluate their immune responses to both the experimental vaccine and to the seasonal vaccine. Each participant will be followed for approximately seven months.

The trial will take place at four NIAID-funded VTEUs. Patients will be enrolled at the Baylor College of Medicine; the University of Iowa in Iowa City; and Cincinnati Children's Hospital Medical Center. Laboratory support will be provided by Saint Louis University. For more information about the study, go to ClinicalTrials.gov, using the identifier NCT03058692.

NIAID conducts and supports research—at NIH, throughout the United States, and worldwide—to study the causes of infectious and immune-mediated diseases, and to develop better means of preventing, diagnosing and treating these illnesses. News releases, fact sheets and other NIAID-related materials are available on the NIAID website.

About the National Institutes of Health (NIH): NIH, the nation's medical research agency, includes 27 Institutes and Centers and is a component of the U.S. Department of Health and Human Services. NIH is the primary federal agency conducting and supporting basic, clinical, and translational medical research, and is investigating the causes, treatments, and cures for both common and rare diseases. For more information about NIH and its programs, visit www.nih.gov.

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http://www.niaid.nih.gov

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Wed, 9 May 2018 15:06:55 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Vance, Meaghan (NIH/NIAID) [C];Haskins, Melinda (NIH/NIAID) [E]
Subject: FW: Senator Markey Request
Importance: High

Please see below. do you want to call Sen Markey from Dallas, pls advise

(b)(6)

Patricia L. Conrad
Special Assistant to the Director

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From: Haskins, Melinda (NIH/NIAID) [E]
Sent: Wednesday, May 09, 2018 11:01 AM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Selgrade, Sara (NIH/NIAID) [E] (b)(6) Billet, Courtney (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Subject: Senator Markey Request
Importance: High

Patty –

Please see the following request from Sen. Markey's office.

Thank you,
Melinda

From: (b)(6)
Sent: Wednesday, May 9, 2018 10:56 AM
To: Haskins, Melinda (NIH/NIAID) [E] (b)(6)

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Cc: (b)(6)
Subject: RE: Update: Flu work briefing

Hi Melinda – hope you're doing well!

I'm reaching out because Sen. Markey would like to call Dr. Fauci to discuss recent developments regarding the universal flu vaccine, specifically the appropriations increase for FY18. I've copied my colleague, Sarah, who would be able to help coordinate a time on the Senator's end. Would you be able to help us arrange that, please?

Thank you so much!
(b)(6)

From: Haskins, Melinda (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, January 24, 2018 7:15 PM
To: (b)(6)
Subject: Re: Update: Flu work briefing

Thanks

Sent from my iPhone

On Jan 24, 2018, at 3:42 PM, (b)(6) wrote:

Running behind – should be reaching out shortly!

From: (b)(6)
Sent: Wednesday, January 24, 2018 10:24 AM
To: 'Haskins, Melinda (NIH/NIAID) [E]' (b)(6)
Cc: (b)(6) Klimczak, Kate (NIH/OD) [E] (b)(6) Berkson, Laura (NIH/OD) [E] (b)(6) Selgrade, Sara (NIH/NIAID) [E] (b)(6)
Subject: RE: Update: Flu work briefing

Thanks so much! Let's aim for 3:30 and will try to shoot you an email if something changes with the timing.

Thank you again for your speedy work on this – we really appreciate it!

From: Haskins, Melinda (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, January 24, 2018 10:11 AM
To: (b)(6)
Cc: (b)(6) Klimczak, Kate (NIH/OD) [E] (b)(6) Berkson, Laura (NIH/OD) [E] (b)(6) Selgrade, Sara (NIH/NIAID) [E] (b)(6)
Subject: Update: Flu work briefing

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Nikki,

Dr. Fauci would be available to speak with Senator Markey after 3 p.m., today. Dr. Fauci can be reached at (b)(6) If this time is inconvenient for the Senator, we will work something out.

Sincerely,

Melinda Haskins
Chief, NIAID OCGR LACMB
Direct: (b)(6)

From: Berkson, Laura (NIH/OD) [E]
Sent: Wednesday, January 24, 2018 9:31 AM
To: (b)(6) Haskins, Melinda (NIH/NIAID) [E]
(b)(6)
Cc: (b)(6) Klimczak, Kate (NIH/OD) [E]
(b)(6)
Subject: RE: Potential flu work briefing?

Hi (b)(6)

No problem – happy to help! I'm looping in my colleague, Melinda Haskins, who works at NIAID and can help set something up.

Best,
Laura

From: (b)(6)
Sent: Tuesday, January 23, 2018 6:45 PM
To: Berkson, Laura (NIH/OD) [E] (b)(6)
Cc: (b)(6)
Subject: RE: Potential flu work briefing?

Hi Laura – so sorry about this, but it looks like Sen. Markey would like to call Dr. Fauci directly to ask some of these questions. Do you have his contact information?

I think he's particularly interested in Dr. Fauci's estimates on how much we'd need to invest to create a universal flu vaccine and develop new/updated models for predicting the flu and producing the vaccine. Happy to hop on the phone tomorrow morning if that would be helpful.

Thanks, and again, apologies for the last minute request. Thanks, Laura!

From: (b)(6)
Sent: Tuesday, January 23, 2018 6:22 PM
To: 'Berkson, Laura (NIH/OD) [E]' (b)(6)
Cc: (b)(6)
Subject: Potential flu work briefing?

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Hi Laura – thanks again for your help with the letter last week!

Following-up on that, do you think it would be possible for us to schedule a call with the relevant folks at NIAID to discuss some of the flu-related work at the agency? We're specifically interested in updates on Dr. Fauci's work to create a universal flu vaccine, and how much the agency thinks it would cost to develop it; what kind of big data or algorithmic work the NIAID is doing, probably in conjunction with other agencies, to better predict the strain in the upcoming flu season; and the agency's work around cell-based flu vaccines.

Do you think this is something we'd be able to schedule for later this week? Please let me know if I can provide any additional information.

Thanks so much!

(b)(6)

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From: Eisinger, Robert (NIH/NIAID) [E]
Sent: Wed, 9 May 2018 14:33:37 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: RE: UN Millennium Development Goals and Sustainable Development Goals
Attachments: UN Sustainable Development Goals and Targets.docx

Please find in the attachment the 17 UN Sustainable Development Goals and Targets. Please note that under Goal 3. Health and well-being is the following:

> "By 2030, end the epidemics of AIDS, tuberculosis, malaria and neglected tropical diseases and combat hepatitis, water-borne diseases and other communicable diseases"

If I can provide any additional information or assistance, please let me know.

Thanks
Bob

From: Eisinger, Robert (NIH/NIAID) [E]
Sent: Wednesday, May 09, 2018 9:19 AM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: UN Millennium Development Goals and Sustainable Development Goals

As requested, please find in the word document the list of 8 UN Millennium Development Goals and 17 UN Sustainable Development Goals. There are specific targets for both lists that I can send separately if it would be useful.
If I can provide any additional information or assistance, please let me know.

Thanks
Bob

Robert W. Eisinger, Ph.D.
Special Assistant for Scientific Projects
Immediate Office of the Director
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 31, Room 7A10A1
31 Center Drive
Bethesda MD 20892
Tel. (b)(6)

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(b)(6)

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UN Development Programme's 17 Sustainable Development Goals (SDGs) (effective 2016 until 2030)

1. **No poverty**

Targets:

- By 2030, reduce at least by half the proportion of men, women and children of all ages living in poverty in all its dimensions according to national definitions
- Implement nationally appropriate social protection systems and measures for all, including floors, and by 2030 achieve substantial coverage of the poor and the vulnerable
- By 2030, ensure that all men and women, in particular the poor and the vulnerable, have equal rights to economic resources, as well as access to basic services, ownership and control over land and other forms of property, inheritance, natural resources, appropriate new technology and financial services, including microfinance
- By 2030, build the resilience of the poor and those in vulnerable situations and reduce their exposure and vulnerability to climate-related extreme events and other economic, social and environmental shocks and disasters
- Ensure significant mobilization of resources from a variety of sources, including through enhanced development cooperation, in order to provide adequate and predictable means for developing countries, in particular least developed countries, to implement programmes and policies to end poverty in all its dimensions
- Create sound policy frameworks at the national, regional and international levels, based on pro-poor and gender-sensitive development strategies, to support accelerated investment in poverty eradication actions

2. **Zero hunger**

Targets:

- By 2030, end hunger and ensure access by all people, in particular the poor and people in vulnerable situations, including infants, to safe, nutritious and sufficient food all year round
- By 2030, end all forms of malnutrition, including achieving, by 2025, the internationally agreed targets on stunting and wasting in children under 5 years of age, and address the nutritional needs of adolescent girls, pregnant and lactating women and older persons
- By 2030, double the agricultural productivity and incomes of small-scale food producers, in particular women, indigenous peoples, family farmers, pastoralists and fishers, including through secure and equal access to land, other productive resources and inputs, knowledge, financial services, markets and opportunities for value addition and non-farm employment
- By 2030, ensure sustainable food production systems and implement resilient agricultural practices that increase productivity and production, that help maintain ecosystems, that strengthen capacity for adaptation to climate change, extreme

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weather, drought, flooding and other disasters and that progressively improve land and soil quality

- By 2020, maintain the genetic diversity of seeds, cultivated plants and farmed and domesticated animals and their related wild species, including through soundly managed and diversified seed and plant banks at the national, regional and international levels, and promote access to and fair and equitable sharing of benefits arising from the utilization of genetic resources and associated traditional knowledge, as internationally agreed
- Increase investment, including through enhanced international cooperation, in rural infrastructure, agricultural research and extension services, technology development and plant and livestock gene banks in order to enhance agricultural productive capacity in developing countries, in particular least developed countries
- Correct and prevent trade restrictions and distortions in world agricultural markets, including through the parallel elimination of all forms of agricultural export subsidies and all export measures with equivalent effect, in accordance with the mandate of the Doha Development Round
- Adopt measures to ensure the proper functioning of food commodity markets and their derivatives and facilitate timely access to market information, including on food reserves, in order to help limit extreme food price volatility

3. Good health and well-being

Targets:

- By 2030, reduce the global maternal mortality ratio to less than 70 per 100,000 live births
- By 2030, end preventable deaths of newborns and children under 5 years of age, with all countries aiming to reduce neonatal mortality to at least as low as 12 per 1,000 live births and under-5 mortality to at least as low as 25 per 1,000 live births
- By 2030, end the epidemics of AIDS, tuberculosis, malaria and neglected tropical diseases and combat hepatitis, water-borne diseases and other communicable diseases
- By 2030, reduce by one third premature mortality from non-communicable diseases through prevention and treatment and promote mental health and well-being
- Strengthen the prevention and treatment of substance abuse, including narcotic drug abuse and harmful use of alcohol
- By 2020, halve the number of global deaths and injuries from road traffic accidents
- By 2030, ensure universal access to sexual and reproductive health-care services, including for family planning, information and education, and the integration of reproductive health into national strategies and programmes
- Achieve universal health coverage, including financial risk protection, access to quality essential health-care services and access to safe, effective, quality and affordable essential medicines and vaccines for all

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- By 2030, substantially reduce the number of deaths and illnesses from hazardous chemicals and air, water and soil pollution and contamination
- Strengthen the implementation of the World Health Organization Framework Convention on Tobacco Control in all countries, as appropriate
- Support the research and development of vaccines and medicines for the communicable and noncommunicable diseases that primarily affect developing countries, provide access to affordable essential medicines and vaccines, in accordance with the Doha Declaration on the TRIPS Agreement and Public Health, which affirms the right of developing countries to use to the full the provisions in the Agreement on Trade Related Aspects of Intellectual Property Rights regarding flexibilities to protect public health, and, in particular, provide access to medicines for all
- Substantially increase health financing and the recruitment, development, training and retention of the health workforce in developing countries, especially in least developed countries and small island developing States
- Strengthen the capacity of all countries, in particular developing countries, for early warning, risk reduction and management of national and global health risks

4. Quality education

Targets:

- By 2030, ensure that all girls and boys complete free, equitable and quality primary and secondary education leading to relevant and Goal-4 effective learning outcomes
- By 2030, ensure that all girls and boys have access to quality early childhood development, care and preprimary education so that they are ready for primary education
- By 2030, ensure equal access for all women and men to affordable and quality technical, vocational and tertiary education, including university
- By 2030, substantially increase the number of youth and adults who have relevant skills, including technical and vocational skills, for employment, decent jobs and entrepreneurship
- By 2030, eliminate gender disparities in education and ensure equal access to all levels of education and vocational training for the vulnerable, including persons with disabilities, indigenous peoples and children in vulnerable situations
- By 2030, ensure that all youth and a substantial proportion of adults, both men and women, achieve literacy and numeracy
- By 2030, ensure that all learners acquire the knowledge and skills needed to promote sustainable development, including, among others, through education for sustainable development and sustainable lifestyles, human rights, gender equality, promotion of a culture of peace and non-violence, global citizenship and appreciation of cultural diversity and of culture's contribution to sustainable development
- Build and upgrade education facilities that are child, disability and gender sensitive and provide safe, nonviolent, inclusive and effective learning environments for all

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- By 2020, substantially expand globally the number of scholarships available to developing countries, in particular least developed countries, small island developing States and African countries, for enrolment in higher education, including vocational training and information and communications technology, technical, engineering and scientific programmes, in developed countries and other developing countries
- By 2030, substantially increase the supply of qualified teachers, including through international cooperation for teacher training in developing countries, especially least developed countries and small island developing states

5. Gender equality

Targets:

- End all forms of discrimination against all women and girls everywhere
- Eliminate all forms of violence against all women and girls in the public and private spheres, including trafficking and sexual and other types of exploitation
- Eliminate all harmful practices, such as child, early and forced marriage and female genital mutilation
- Recognize and value unpaid care and domestic work through the provision of public services, infrastructure and social protection policies and the promotion of shared responsibility within the household and the family as nationally appropriate
- Ensure women's full and effective participation and equal opportunities for leadership at all levels of decisionmaking in political, economic and public life
- Ensure universal access to sexual and reproductive health and reproductive rights as agreed in accordance with the Programme of Action of the International Conference on Population and Development and the Beijing Platform for Action and the outcome documents of their review conferences
- Undertake reforms to give women equal rights to economic resources, as well as access to ownership and control over land and other forms of property, financial services, inheritance and natural resources, in accordance with national laws
- Enhance the use of enabling technology, in particular information and communications technology, to promote the empowerment of women
- Adopt and strengthen sound policies and enforceable legislation for the promotion of gender equality and the empowerment of all women and girls at all levels

6. Clean water and sanitation

Targets:

- By 2030, achieve universal and equitable access to safe and affordable drinking water for all
- By 2030, achieve access to adequate and equitable sanitation and hygiene for all and end open defecation, paying special attention to the needs of women and girls and those in vulnerable situations
- By 2030, improve water quality by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion

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of untreated wastewater and substantially increasing recycling and safe reuse globally

- By 2030, substantially increase water-use efficiency across all sectors and ensure sustainable withdrawals and supply of freshwater to address water scarcity and substantially reduce the number of people suffering from water scarcity
- By 2030, implement integrated water resources management at all levels, including through transboundary cooperation as appropriate
- By 2020, protect and restore water-related ecosystems, including mountains, forests, wetlands, rivers, aquifers and lakes
- By 2030, expand international cooperation and capacity-building support to developing countries in water- and sanitation-related activities and programmes, including water harvesting, desalination, water efficiency, wastewater treatment, recycling and reuse technologies
- Support and strengthen the participation of local communities in improving water and sanitation management

7. Affordable and clean energy

Targets:

- By 2030, ensure universal access to affordable, reliable and modern energy services
- By 2030, increase substantially the share of renewable energy in the global energy mix
- By 2030, double the global rate of improvement in energy efficiency
- By 2030, enhance international cooperation to facilitate access to clean energy research and technology, including renewable energy, energy efficiency and advanced and cleaner fossil-fuel technology, and promote investment in energy infrastructure and clean energy technology
- By 2030, expand infrastructure and upgrade technology for supplying modern and sustainable energy services for all in developing countries, in particular least developed countries, small island developing States, and land-locked developing countries, in accordance with their respective programmes of support

8. Decent work and economic growth

Targets:

- Sustain per capita economic growth in accordance with national circumstances and, in particular, at least 7 per cent gross domestic product growth per annum in the least developed countries
- Achieve higher levels of economic productivity through diversification, technological upgrading and innovation, including through a focus on high-value added and labour-intensive sectors
- Promote development-oriented policies that support productive activities, decent job creation, entrepreneurship, creativity and innovation, and encourage the formalization and growth of micro-, small- and medium-sized enterprises, including through access to financial services

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- Improve progressively, through 2030, global resource efficiency in consumption and production and endeavour to decouple economic growth from environmental degradation, in accordance with the 10-year framework of programmes on sustainable consumption and production, with developed countries taking the lead
- By 2030, achieve full and productive employment and decent work for all women and men, including for young people and persons with disabilities, and equal pay for work of equal value
- By 2020, substantially reduce the proportion of youth not in employment, education or training
- Take immediate and effective measures to eradicate forced labour, end modern slavery and human trafficking and secure the prohibition and elimination of the worst forms of child labour, including recruitment and use of child soldiers, and by 2025 end child labour in all its forms
- Protect labour rights and promote safe and secure working environments for all workers, including migrant workers, in particular women migrants, and those in precarious employment
- By 2030, devise and implement policies to promote sustainable tourism that creates jobs and promotes local culture and products
- Strengthen the capacity of domestic financial institutions to encourage and expand access to banking, insurance and financial services for all
- Increase Aid for Trade support for developing countries, in particular least developed countries, including through the Enhanced Integrated Framework for Trade-Related Technical Assistance to Least Developed Countries
- By 2020, develop and operationalize a global strategy for youth employment and implement the Global Jobs Pact of the International Labour Organization

9. Industry, innovation, and infrastructure

Targets:

- Develop quality, reliable, sustainable and resilient infrastructure, including regional and transborder infrastructure, to support economic development and human well-being, with a focus on affordable and equitable access for all
- Promote inclusive and sustainable industrialization and, by 2030, significantly raise industry's share of employment and gross domestic product, in line with national circumstances, and double its share in least developed countries
- Increase the access of small-scale industrial and other enterprises, in particular in developing countries, to financial services, including affordable credit, and their integration into value chains and markets
- By 2030, upgrade infrastructure and retrofit industries to make them sustainable, with increased resource-use efficiency and greater adoption of clean and environmentally sound technologies and industrial processes, with all countries taking action in accordance with their respective capabilities
- Enhance scientific research, upgrade the technological capabilities of industrial sectors in all countries, in particular developing countries, including, by 2030, encouraging innovation and substantially increasing the number of research and

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development workers per 1 million people and public and private research and development spending

- Facilitate sustainable and resilient infrastructure development in developing countries through enhanced financial, technological and technical support to African countries, least developed countries, landlocked developing countries and small island developing States 18
- Support domestic technology development, research and innovation in developing countries, including by ensuring a conducive policy environment for, inter alia, industrial diversification and value addition to commodities
- Significantly increase access to information and communications technology and strive to provide universal and affordable access to the Internet in least developed countries by 2020

10. Reduced inequalities

Targets:

- By 2030, progressively achieve and sustain income growth of the bottom 40 per cent of the population at a rate higher than the national average
- By 2030, empower and promote the social, economic and political inclusion of all, irrespective of age, sex, disability, race, ethnicity, origin, religion or economic or other status
- Ensure equal opportunity and reduce inequalities of outcome, including by eliminating discriminatory laws, policies and practices and promoting appropriate legislation, policies and action in this regard
- Adopt policies, especially fiscal, wage and social protection policies, and progressively achieve greater equality
- Improve the regulation and monitoring of global financial markets and institutions and strengthen the implementation of such regulations
- Ensure enhanced representation and voice for developing countries in decision-making in global international economic and financial institutions in order to deliver more effective, credible, accountable and legitimate institutions
- Facilitate orderly, safe, regular and responsible migration and mobility of people, including through the implementation of planned and well-managed migration policies
- Implement the principle of special and differential treatment for developing countries, in particular least developed countries, in accordance with World Trade Organization agreements
- Encourage official development assistance and financial flows, including foreign direct investment, to States where the need is greatest, in particular least developed countries, African countries, small island developing States and landlocked developing countries, in accordance with their national plans and programmes
- By 2030, reduce to less than 3 per cent the transaction costs of migrant remittances and eliminate remittance corridors with costs higher than 5 per cent

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11. Sustainable cities and communities

Targets:

- By 2030, ensure access for all to adequate, safe and affordable housing and basic services and upgrade slums
- By 2030, provide access to safe, affordable, accessible and sustainable transport systems for all, improving road safety, notably by expanding public transport, with special attention to the needs of those in vulnerable situations, women, children, persons with disabilities and older persons
- By 2030, enhance inclusive and sustainable urbanization and capacity for participatory, integrated and sustainable human settlement planning and management in all countries
- Strengthen efforts to protect and safeguard the world's cultural and natural heritage
- By 2030, significantly reduce the number of deaths and the number of people affected and substantially decrease the direct economic losses relative to global gross domestic product caused by disasters, including water-related disasters, with a focus on protecting the poor and people in vulnerable situations
- By 2030, reduce the adverse per capita environmental impact of cities, including by paying special attention to air quality and municipal and other waste management
- By 2030, provide universal access to safe, inclusive and accessible, green and public spaces, in particular for women and children, older persons and persons with disabilities
- Support positive economic, social and environmental links between urban, peri-urban and rural areas by strengthening national and regional development planning
- By 2020, substantially increase the number of cities and human settlements adopting and implementing integrated policies and plans towards inclusion, resource efficiency, mitigation and adaptation to climate change, resilience to disasters, and develop and implement, in line with the Sendai Framework for Disaster Risk Reduction 2015-2030, holistic disaster risk management at all levels
- Support least developed countries, including through financial and technical assistance, in building sustainable and resilient buildings utilizing local materials

12. Responsible consumption and production

Targets:

- Implement the 10-year framework of programmes on sustainable consumption and production, all countries taking action, with developed countries taking the lead, taking into account the development and capabilities of developing countries
- By 2030, achieve the sustainable management and efficient use of natural resources
- By 2030, halve per capita global food waste at the retail and consumer levels and reduce food losses along production and supply chains, including post-harvest losses
- By 2020, achieve the environmentally sound management of chemicals and all wastes throughout their life cycle, in accordance with agreed international frameworks, and

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significantly reduce their release to air, water and soil in order to minimize their adverse impacts on human health and the environment

- By 2030, substantially reduce waste generation through prevention, reduction, recycling and reuse
- Encourage companies, especially large and transnational companies, to adopt sustainable practices and to integrate sustainability information into their reporting cycle
- Promote public procurement practices that are sustainable, in accordance with national policies and priorities
- By 2030, ensure that people everywhere have the relevant information and awareness for sustainable development and lifestyles in harmony with nature
- Support developing countries to strengthen their scientific and technological capacity to move towards more sustainable patterns of consumption and production
- Develop and implement tools to monitor sustainable development impacts for sustainable tourism that creates jobs and promotes local culture and products
- Rationalize inefficient fossil-fuel subsidies that encourage wasteful consumption by removing market distortions, in accordance with national circumstances, including by restructuring taxation and phasing out those harmful subsidies, where they exist, to reflect their environmental impacts, taking fully into account the specific needs and conditions of developing countries and minimizing the possible adverse impacts on their development in a manner that protects the poor and the affected communities

13. Climate action

Targets:

- Strengthen resilience and adaptive capacity to climate-related hazards and natural disasters in all countries
- Integrate climate change measures into national policies, strategies and planning
- Improve education, awareness-raising and human and institutional capacity on climate change mitigation, adaptation, impact reduction and early warning
- Implement the commitment undertaken by developed-country parties to the United Nations Framework Convention on Climate Change to a goal of mobilizing jointly $100 billion annually by 2020 from all sources to address the needs of developing countries in the context of meaningful mitigation actions and transparency on implementation and fully operationalize the Green Climate Fund through its capitalization as soon as possible
- Promote mechanisms for raising capacity for effective climate change-related planning and management in least developed countries and small island developing States, including focusing on women, youth and local and marginalized communities

14. Life below water

Targets:

- By 2025, prevent and significantly reduce marine pollution of all kinds, in particular from land-based activities, including marine debris and nutrient pollution

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- By 2020, sustainably manage and protect marine and coastal ecosystems to avoid significant adverse impacts, including by strengthening their resilience, and take action for their restoration in order to achieve healthy and productive oceans
- Minimize and address the impacts of ocean acidification, including through enhanced scientific cooperation at all levels
- By 2020, effectively regulate harvesting and end overfishing, illegal, unreported and unregulated fishing and destructive fishing practices and implement science-based management plans, in order to restore fish stocks in the shortest time feasible, at least to levels that can produce maximum sustainable yield as determined by their biological characteristics
- By 2020, conserve at least 10 per cent of coastal and marine areas, consistent with national and international law and based on the best available scientific information
- By 2020, prohibit certain forms of fisheries subsidies which contribute to overcapacity and overfishing, eliminate subsidies that contribute to illegal, unreported and unregulated fishing and refrain from introducing new such subsidies, recognizing that appropriate and effective special and differential treatment for developing and least developed countries should be an integral part of the World Trade Organization fisheries subsidies negotiation
- By 2030, increase the economic benefits to Small Island developing States and least developed countries from the sustainable use of marine resources, including through sustainable management of fisheries, aquaculture and tourism
- Increase scientific knowledge, develop research capacity and transfer marine technology, taking into account the Intergovernmental Oceanographic Commission Criteria and Guidelines on the Transfer of Marine Technology, in order to improve ocean health and to enhance the contribution of marine biodiversity to the development of developing countries, in particular small island developing States and least developed countries
- Provide access for small-scale artisanal fishers to marine resources and markets
- Enhance the conservation and sustainable use of oceans and their resources by implementing international law as reflected in UNCLOS, which provides the legal framework for the conservation and sustainable use of oceans and their resources, as recalled in paragraph 158 of The Future We Want

15. Life on land

Targets:

- By 2020, ensure the conservation, restoration and sustainable use of terrestrial and inland freshwater ecosystems and their services, in particular forests, wetlands, mountains and drylands, in line with obligations under international agreements
- By 2020, promote the implementation of sustainable management of all types of forests, halt deforestation, restore degraded forests and substantially increase afforestation and reforestation globally
- By 2030, combat desertification, restore degraded land and soil, including land affected by desertification, drought and floods, and strive to achieve a land degradation-neutral world

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- By 2030, ensure the conservation of mountain ecosystems, including their biodiversity, in order to enhance their capacity to provide benefits that are essential for sustainable development
- Take urgent and significant action to reduce the degradation of natural habitats, halt the loss of biodiversity and, by 2020, protect and prevent the extinction of threatened species
- Promote fair and equitable sharing of the benefits arising from the utilization of genetic resources and promote appropriate access to such resources, as internationally agreed
- Take urgent action to end poaching and trafficking of protected species of flora and fauna and address both demand and supply of illegal wildlife products
- By 2020, introduce measures to prevent the introduction and significantly reduce the impact of invasive alien species on land and water ecosystems and control or eradicate the priority species
- By 2020, integrate ecosystem and biodiversity values into national and local planning, development processes, poverty reduction strategies and accounts
- Mobilize and significantly increase financial resources from all sources to conserve and sustainably use biodiversity and ecosystems
- Mobilize significant resources from all sources and at all levels to finance sustainable forest management and provide adequate incentives to developing countries to advance such management, including for conservation and reforestation
- Enhance global support for efforts to combat poaching and trafficking of protected species, including by increasing the capacity of local communities to pursue sustainable livelihood opportunities

16. Peace, justice and strong institutions

Targets:

- Significantly reduce all forms of violence and related death rates everywhere
- End abuse, exploitation, trafficking and all forms of violence against and torture of children
- Promote the rule of law at the national and international levels and ensure equal access to justice for all
- By 2030, significantly reduce illicit financial and arms flows, strengthen the recovery and return of stolen assets and combat all forms of organized crime
- Substantially reduce corruption and bribery in all their forms
- Develop effective, accountable and transparent institutions at all levels
- Ensure responsive, inclusive, participatory and representative decision-making at all levels
- Broaden and strengthen the participation of developing countries in the institutions of global governance
- By 2030, provide legal identity for all, including birth registration
- Ensure public access to information and protect fundamental freedoms, in accordance with national legislation and international agreements

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- Strengthen relevant national institutions, including through international cooperation, for building capacity at all levels, in particular in developing countries, to prevent violence and combat terrorism and crime
- Promote and enforce non-discriminatory laws and policies for sustainable development

17. Partnerships for the goals

Targets:

Finance

- Strengthen domestic resource mobilization, including through international support to developing countries, to improve domestic capacity for tax and other revenue collection
- Developed countries to implement fully their official development assistance commitments, including the commitment by many developed countries to achieve the target of 0.7 per cent of ODA/GNI to developing countries and 0.15 to 0.20 per cent of ODA/GNI to least developed countries ODA providers are encouraged to consider setting a target to provide at least 0.20 per cent of ODA/GNI to least developed countries
- Mobilize additional financial resources for developing countries from multiple sources
- Assist developing countries in attaining long-term debt sustainability through coordinated policies aimed at fostering debt financing, debt relief and debt restructuring, as appropriate, and address the external debt of highly indebted poor countries to reduce debt distress
- Adopt and implement investment promotion regimes for least developed countries

Technology

- Enhance North-South, South-South and triangular regional and international cooperation on and access to science, technology and innovation and enhance knowledge sharing on mutually agreed terms, including through improved coordination among existing mechanisms, in particular at the United Nations level, and through a global technology facilitation mechanism
- Promote the development, transfer, dissemination and diffusion of environmentally sound technologies to developing countries on favourable terms, including on concessional and preferential terms, as mutually agreed
- Fully operationalize the technology bank and science, technology and innovation capacity-building mechanism for least developed countries by 2017 and enhance the use of enabling technology, in particular information and communications technology

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Capacity building

- Enhance international support for implementing effective and targeted capacity-building in developing countries to support national plans to implement all the sustainable development goals, including through North-South, South-South and triangular cooperation

Trade

- Promote a universal, rules-based, open, non-discriminatory and equitable multilateral trading system under the World Trade Organization, including through the conclusion of negotiations under its Doha Development Agenda
- Significantly increase the exports of developing countries, in particular with a view to doubling the least developed countries' share of global exports by 2020
- Realize timely implementation of duty-free and quota-free market access on a lasting basis for all least developed countries, consistent with World Trade Organization decisions, including by ensuring that preferential rules of origin applicable to imports from least developed countries are transparent and simple, and contribute to facilitating market access

Systemic issues

Policy and institutional coherence

- Enhance global macroeconomic stability, including through policy coordination and policy coherence
- Enhance policy coherence for sustainable development
- Respect each country's policy space and leadership to establish and implement policies for poverty eradication and sustainable development

Multi-stakeholder partnerships

- Enhance the global partnership for sustainable development, complemented by multi-stakeholder partnerships that mobilize and share knowledge, expertise, technology and financial resources, to support the achievement of the sustainable development goals in all countries, in particular developing countries
- Encourage and promote effective public, public-private and civil society partnerships, building on the experience and resourcing strategies of partnerships

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Data, monitoring and accountability

- By 2020, enhance capacity-building support to developing countries, including for least developed countries and small island developing States, to increase significantly the availability of high-quality, timely and reliable data disaggregated by income, gender, age, race, ethnicity, migratory status, disability, geographic location and other characteristics relevant in national contexts
- By 2030, build on existing initiatives to develop measurements of progress on sustainable development that complement gross domestic product, and support statistical capacity-building in developing countries

Ref. http://www.undp.org/content/undp/en/home/sustainable-development-goals.html

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Wed, 9 May 2018 13:32:32 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Vance, Meaghan (NIH/NIAID) [C]
Subject: per your question about cancelling DIV DIR on Thursday

carol and Hugh would ask to keep it on – the biggest topic is universal flu presentations from both DMID and DAIT\

pls advise

Patricia L. Conrad
Public Health Analyst and
Special Assistant to the Director
National Institute of Allergy and Infectious Diseases
The National Institutes of Health
31 Center Drive, MSC 2520 - Room 7A03
Bethesda, Maryland 20892
(b)(6)
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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Tue, 8 May 2018 15:32:51 +0000
To: Fauci, Anthony (NIH/NIAID) [E];Paules, Catharine (NIH/NIAID) [E]
Subject: Pre brief for Arbovirus meeting at GWU on 5-6 June (Cat)
Attachments: June 5th arbovirus talk, RE: Prebrief for GW

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From: Paules, Catharine (NIH/NIAID) [E]
Sent: Tue, 8 May 2018 15:19:11 +0000
To: Vance, Meaghan (NIH/NIAID) [C]
Cc: Conrad, Patricia (NIH/NIAID) [E]
Subject: June 5th arbovirus talk

Hi Meaghan,
Is there time on the calendar later this week to discuss this talk? Would need a half hour. Thanks, Cat

---

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Tuesday, May 08, 2018 10:17 AM
To: Paules, Catharine (NIH/NIAID) [E] (b)(6)
Cc: Folkers, Greg (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Subject: FW: on behalf of Dr. Anthony Fauci

Below are details for June 5 talk.

Meaghan – pls add copy to folder thx.

Patricia L. Conrad
Special Assistant to the Director

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From: Jon Andrus (b)(6)
Sent: Tuesday, May 08, 2018 10:09 AM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Halley Petersen-Jobsis (b)(6) Lynn Goldman (b)(6)
Subject: Fwd: on behalf of Dr. Anthony Fauci

Dear Pat,

I just received your message. I am currently in (b)(6) doing work for the global measles rubella elimination initiative.

Please see my responses in parentheses to your questions below. I am copying Halley Petersen-Jobsis from the GWU who will know the exact name of the conference hall at the Milken Institute for Global Health on George Washington Circle, next to the GWU Hospital. She will also send the agenda and be your prime logistical contact.

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Thanks for coordinating the visit of Dr. Fauci. I am looking forward to seeing him, I know Lynn is too!

Also, do not hesitate to contact me on my cell (b)(6) if you need any additional assistance. I will be back in WDC the evening of 17 May.

Please confirm that you received this. Connectivity is occasionally an issue.

All the best,

Jon

---------- Forwarded message ----------
From: **Lynn Goldman** (b)(6)
Date: Tue, May 8, 2018 at 8:18 PM
Subject: Fwd: on behalf of Dr. Anthony Fauci
To: Jon Andrus (b)(6) Jon Andrus (b)(6) Jon Andrus (b)(6)
Cc: Natasha Nathan (b)(6)

Dear Jon,

The NIAID is sending out a search party to find you to respond to their email about the arbovirus meeting. Please confirm that you have received this/can get back to them.

Truly yours,
Lynn
---------- Forwarded message ----------
From: **Conrad, Patricia (NIH/NIAID) [E]** (b)(6)
Date: Tue, May 8, 2018 at 9:13 AM
Subject: on behalf of Dr. Anthony Fauci
To: (b)(6) (b)(6)

Good morning:

I am hoping you can help me with the below email. Can you please direct me to the conference coordinator for the below lecture. We have not heard back from Dr. Andrus.

Thank you.

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Patricia L. Conrad
Public Health Analyst and
Special Assistant to the Director
National Institute of Allergy and Infectious Diseases
The National Institutes of Health
31 Center Drive, MSC 2520 - Room 7A03
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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Monday, May 07, 2018 11:40 AM
To: (b)(6) (b)(6)
Subject: RE: Arbovirus meeting at GWU on 5-6 June

Good morning Dr. Andrus:

I am touching base to get more detail on your event that Dr. Fauci will speak at on June 5th. We would like to prepare his remarks. Our questions include:

1. Exact time for the lecture – we are holding that morning. (8:45 am)
2. Room and building location for the event (See above. Halley will provide name.)
3. How long you would like Dr. Fauci to speak for and if there will be a q and a after (We would like Dr. Fauci to speak for 25 minutes with 5 minutes to field a few questions. I know he is busy, so he could depart after a few questions. I will be moderating. We are planning to have webcast - Halley can confirm)

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4. Who is in the audience and how many? So that we can tailor his remarks (The key audience is a group of 25 global experts, mostly from Latin American and the Caribbean, who will be writing a white paper to inform policy on the prevention and control of Aedes transmitted Arboviruses in Latin America and the Caribbean. PAHO is fully engaged and appreciates this effort. In addition, there will be public health experts attending from the PAHO general staff, other academics from LAC and the US, and other public health partners in the WDC area. Finally, the room of 220 occupancy will also have in attendance graduate students and other interested faculty.)
5. Please confirm that slides are appropriate – Dr. Fauci will use powerpoint. We ask that you provide a PC laptop at the podium so that he can closely see and advance his own slides (Yes, PPT presentation is welcomed and we will be sure he access to a laptop at the lectern.)
6. Is there a detailed agenda you can send with who else will be speaking (I will ask Halley by copy of this to her to send you the detailed agenda, since I am Thailand and will not be returning until 17 May.)
7. Please send the onsite logistical contacts. (Halley will be your prime logistical contact)

Thank you and I look forward to hearing from you.

Best,
-patty

Patricia L. Conrad
Special Assistant to the Director

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Tuesday, January 09, 2018 3:55 PM
To: (b)(6) (b)(6)
Subject: FW: Arbovirus meeting at GWU on 5-6 June

Dr. Andrus:

Dr. Fauci asked that I respond to your email on his behalf – he wanted me to thank you for contacting him. He would be happy to deliver the keynote at your meeting on June 5th. I will be the point of contact for his logistics. Please let me know is you need anything else at this time. Otherwise, we can just touch base closer to the event date.

Best,
-patty

Patricia L. Conrad
Special Assistant to the Director

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Begin forwarded message:

From: Jon Andrus (b)(6)
Date: January 9, 2018 at 10:59:11 AM EST
To: (b)(6)
Subject: Arbovirus meeting at GWU on 5-6 June
Reply-To: (b)(6)

Dear Tony,

Hope all is well.

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Dean Lynn Goldman of GWU and I are organizing a scientific symposium on prevention and control of arboviruses 5-6 June at GWU in WDC. I am writing to see if you would be willing to provide the keynote address.

When I was Deputy Director of PAHO, you gave an outstanding presentation in celebration of the lifetime contribution to global health of Dr. Ciro de Quadros. We organized that event a few months (b)(6) At the time he whispered in my ear, "Just what the doctor ordered." For that I am eternally grateful for your contribution. I know Ciro was. I also remember distinctly the tremendous help you provided us when (b)(6) on his way to the PAHO TAG meeting, taking my calls at all hours to assist the family.

It would be an honor and pleasure to be able to work with you again on an important issue if you are available to give the keynote speech the morning of 5 June. Thanks for your time and consideration.

All the best,

Jon

Jon Kim Andrus, MD
Adjunct Professor Global Health
Milken Institute of Public Health
George Washington University

Adjoint Professor and Director of the Department of Vaccines and Immunization
Center for Global Health
University of Colorado

--

Lynn R. Goldman, MD, MS, MPH
Michael and Lori Milken Dean, Milken Institute School of Public Health,
George Washington University
(b)(6)

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From: Paules, Catharine (NIH/NIAID) [E]
Sent: Thu, 10 May 2018 16:20:28 +0000
To: Vance, Meaghan (NIH/NIAID) [C]
Cc: Conrad, Patricia (NIH/NIAID) [E]
Subject: RE: Prebrief for GW

Sure can. Thanks very much

From: Vance, Meaghan (NIH/NIAID) [C]
Sent: Thursday, May 10, 2018 12:20 PM
To: Paules, Catharine (NIH/NIAID) [E] (b)(6)
Cc: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: RE: Prebrief for GW

We might be able to do 2pm today, but it will depend on when ASF returns from VRC. Can you be on standby?

From: Paules, Catharine (NIH/NIAID) [E]
Sent: Thursday, May 10, 2018 11:13 AM
To: Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Cc: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: Prebrief for GW

Hi Meaghan,
Any chance of squeezing it in today. Theres a chance I might have to stay home w Henry tomorrow (he was still febrile this morning). Today, I have a meeting w JJ 1:30-2:00 and a meeting w John and Emily 3:40-4:00 and otherwise am open. If not I could call him tomorrow if I have to stay home. Thanks, Cat

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Tue, 8 May 2018 19:10:33 +0000
To: (b)(6)
Cc: Conrad, Patricia (NIH/NIAID) [E]
Subject: FW: David Cooper Remembrance

Cliff:
Among the many things that we need to talk about on your return, here is one.
Thanks,
ASF

From: Daniel Tarantola (b)(6)
Sent: Monday, May 07, 2018 9:01 AM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Auchincloss, Hugh (NIH/NIAID) [E] (b)(6) Alfredo Morabia (b)(6) Lane, Cliff (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6) Farzana Kapadia (b)(6) Brian Selzer (b)(6)
Subject: Re: David Cooper Remembrance

Tony,
This is wonderful and both Alfredo Morabia (*AJPH* Editor in Chief) and I are looking forward to your contribution. The authorship or co-authorship is left up to your decision. We are very glad to learn that Clifford Lane could recall his friendship and close collaboration with David. An August delivery date for the piece would be great. If you have a high resolution picture of David in your and Clifford's company, please send it along with your editorial.
Best wishes,
Daniel

Prof. Daniel Tarantola M.D.
International and Global Health Consultant
15bis, Chemin de Valavran
01210 Ferney-Voltaire
France
Tel. (b)(6)
email: (b)(6)
Associate Editor, The American Journal of Public Health
Fellow of the Royal Society of Medicine
Adjunct Research Professor
Keck School of Medicine
University of Southern California
Los Angeles CA 90033-9022, USA

On Mon, May 7, 2018 at 1:35 PM, Fauci, Anthony (NIH/NIAID) [E] (b)(6) wrote:
Daniel:

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Thanks for the note. I Would be happy to do this. My close associate and Deputy Director here at NIAID, Dr. H. Clifford Lane, was not only a colleague of David's, but also his close personal friend. I would like to include Cliff as a co-author since this would make the editorial more complete and compelling. We can have it for you by August following the International AIDS Meeting, or even sooner depending on other commitments. I appreciate your asking me.
Best regards,
Tony

From: Daniel Tarantola (b)(6)
Sent: Monday, May 07, 2018 6:48 AM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Auchincloss, Hugh (NIH/NIAID) [E] (b)(6) Alfredo Morabia (b)(6)
Subject: David Cooper Remembrance

Dear Tony,

The world HIV community (b)(6) but unfortunately, many will not have the opportunity to attend the event that will be held in Sydney to celebrate him and his admirable work.

I assume that you must have been approached by many to write (b)(6) as you were both world leaders in HIV research and knew each other very well.

On behalf of the *American Journal of Public Health* (AJPH), I am taking the chance of calling on your good will. This journal does not publish obituaries but creates space occasionally for invited editorials building on personal accounts of past interactions with the defunct, highlighting his/her personal characteristics, professional accomplishments and contributions to science, society and humanity.

I wonder if you would agree to submit such a piece to the AJPH and, if so, what time frame would suit you best. Editorials are typically 1200 words of text long, with subheadings, 1 small table or figure if necessary, and no more than 7 references.

I hope you will accept this invitation.

Best regards,

Daniel

Daniel Tarantola M.D.
International and Global Health Consultant
15bis, Chemin de Valavran
01210 Ferney-Voltaire
France
Tel (b)(6)

AMERICAN OVERSIGHT

email: (b)(6)

Associate Editor, The American Journal of Public Health
Fellow of the Royal Society of Medicine
Adjunct Research Professor
Keck School of Medicine
University of Southern California

Los Angeles CA 90033-9022, USA

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From: Paules, Catharine (NIH/NIAID) [E]
Sent: Tue, 8 May 2018 16:13:48 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Conrad, Patricia (NIH/NIAID) [E]
Subject: Commentary for the Journal of Immunology

Here is a draft for Adrian

Dear Adrian,
Dr. Fauci asked me to reach out to you to see if you might be interested in working with us, as a co-author, on a commentary for a topical review issue—"Immunity to Influenza: A Moving Target" for *The Journal of Immunology.* The commentary would be approximately 2000 words and would need to be submitted by September 1, 2018. I would do the bulk of the drafting but would much appreciate your expertise on framing the manuscript and editing. If you are interested Dr. Fauci would like to set up a brief meeting to discuss in the next week or two. Thanks very much, Cat

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From: Barbara Bush
Sent: Tue, 8 May 2018 11:23:37 -0400
To: Fauci, Anthony (NIH/NIAID)
(b)(6)
(b)(6)
(b)(6) Mark Dybul;Yvette Umwali
Cc: Sullivan, Kevin;Walker, Annie;Prince, Emma;Mera Boulus;Brittany Cesarini
Subject: PEPFAR 15 Anniversary event at Bush Center tomorrow
Attachments: 05.09.18 Schedule for Panelists.docx, Lazarus Effect Engage Moderator Questions.docx

Hi wonderful panelists,
We are very much looking forward to seeing you all in Dallas tomorrow.
Attached is both a schedule for the evening, along with the questions I'll be prepared to ask. Because each panel is relatively short, I'll bounce around to make sure we hear from as many folks as possible and cover as many points as possible. We are lucky to have clips from the fab documentary Bending the Arc (featuring many of you) to illustrate the points we'll be discussing.
Please let me know if you have any questions. Can't wait to see you all tomorrow!
Love,
Barbara

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Engage

Presented by HIGHLAND CAPITAL MANAGEMENT

The Lazarus Effect, 15 Years Later
Wednesday, May 9, 2018
George W. Bush Presidential Center

SPEAKER SCHEDULE

5:00 p.m. Premier Transportation picks up speakers at the Highland drops off at the Bush Institute
5:30 p.m. **Speaker Walk Through**
5:30 p.m. Doors open
Registration— Founding Donor Wall, Museum
Museum Tours
Cash Bar— Institute Cross Hall
Museum Pop Up Shop open in Institute Alcove
6:10 p.m. Program participants proceed to green room to receive lavalier microphone
6:25 p.m. Auditorium doors close
6:28 p.m. *Engage Video plays*

Off-stage announce of President George W. Bush

6:30 p.m. **Welcome Remarks and Introduction**
President George W. Bush

6:45 p.m. **Panel One**
Barbara P. Bush, Co-founder and Board Member, Global Health Corps
Dr. Agnes Binagwaho, Former Rwandan Minister of Health
Ambassador Mark Dybul, M.D.
Michael Gerson, Washington Post

7:25 p.m. **Panel Two**
Barbara P. Bush, Co-founder and Board Member, Global Health Corps
Dr. Paul Farmer, Partner in Health
Dr. Anthony Fauci, National Institute of Health
Senator Lindsey Graham, (R-South Carolina)
Favorite Iradukunda, Global Health Alumni

8:00 p.m. **Closing Remarks**
Ken Hersh, President and CEO, George W. Bush Presidential Center

8:05 p.m. Event Concludes

8:10 p.m. Program Participant Dinner
Presidential Reception Hall

9:30 p.m. Premier Transportation to pick up speakers at Bush Institute and drop off at Highland Hotel

Contact Information:

Annie Walker (214) 864-6246 Emma Prince (214) 675-2106 Kevin Sullivan (703) 343-5757

AMERICAN OVERSIGHT

May 8, 2018

The Lazarus Effect: 15 Years Later // Suggested Questions

Panel 1 (6:45pm): Discuss the origin of PEPFAR: The largest international health initiative in history for a single disease which saved lives and changed the paradigm. Participants:

- Former Rwandan Minister of Health **Dr. Agnes Binagwaho**
- Key architect of PEPFAR **Ambassador Mark Dybul, M.D.**
- *Washington Post* columnist **Michael Gerson**

Suggested Questions / Framework:

[Note: Will adjust if Bridget Chisenga's video survivor testimonial is added to the program]

Begin with Mark:

Q: Take us back to 2002. Can you walk us through what was happening with the AIDs epidemic in Africa…and how global leaders were attempting to address it with the Global Fund?

VIDEO: ANDREW NATSIOS OF USAID "They don't have watches…" (RT=42 SECONDS)

Q: When did you first hear that dad wanted the United States to take the lead and do something bold and ambitious – and what happened next?

For Gerson:

Q: In November 2002 there was a pivotal meeting the Oval Office when dad told you and his senior team about the bold vision he wanted to announce in the State of the Union address. Can you describe that meeting for us and what happened next?

VIDEO: PRES BUSH 2003 SOTU PEPFAR ANNOUNCEMENT (RT=3:11)

For Dr. Agnes:

Q: While all this was taking place in Washington, you were a pediatrician in Rwanda, working with HIV patients in clinics and public hospitals. What did the PEPFAR announcement mean to the people of Rwanda?

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May 8, 2018

VIDEO: From BTA - Paul Farmer in Haiti with patient who could have been cured if meds were available (RT=1:28)

For Mark and Mike:

Q: Making the announcement was just the beginning. How did dad build bipartisan support for PEPFAR on the Hill and move the bureaucracy to get it done?

For Dr. Agnes:

Q: Perhaps one of the transformational, animating principles and legacies of PEPFAR was the shift from a paternalistic approach to one of true partnership. Rwandan President Paul Kagame said, "This is the first time someone respected us enough to hold us accountable." As an African medical practitioner, how did you feel about the accountability component?

For Mark:

Q: Are there other lasting legacies of PEPFAR beyond the shift from paternalism to partnership we just discussed with Agnes?

For Mike:

Q: Our next panel will talk about the state of affairs in Africa today…and the prospect of controlling, and ultimately we hope, ending the HIV epidemic. You've described PEPFAR as taking people from hopeless to hopeful. Let's conclude our discussion by having each of you offer a reflection on what your work in those early days means to you today…and how hopeful you are for the impact of PEPFAR to live on in sub-Saharan Africa.

Mike?

Dr. Agnes?

Mark, we'll give you the last word

Panel 2 (7:25pm): The state of health in Africa today…and looking forward, getting to the end of the epidemic. Participants:

- Key architect of PEPFAR **Dr. Anthony Fauci**
- Partner In Health's **Dr. Paul Farmer**

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- **Favorite "Favo" Iradukunda**, PhD student, College of Nursing at U-Mass Amherst; Global Health Corps alum

- **Senator Lindsey Graham** (R-South Carolina)

BPB: Thank you Mark, Mike and Dr. Agnes for being here…as we re-set the stage for our next panel, here's a short clip from the film, Bending the Arc…

[Play Bending the Arc "before and after" clip during the set change]

BPB: That was incredible. One of the filmmakers of Bending the Arc, **Cori Shepherd Stern**, is with us here tonight (confirm)…welcome Cori and thank you for helping tell the story of PEPFAR through this powerful film (applause)

BPB: Intro second panel (bio bullets to come)

For Tony:

Q: What is the current state of the HIV pandemic and what does the future look like?

Note: This question gives Tony the chance to explain that there is a big difference between having the tools available and being able to end it…that we can't misunderstand this point in the U.S. and walk away – perfect set up for Sen. Graham)

For Sen. Graham:

Q: Senator Graham – thank you for your remarkable leadership to make sure the funding for PEFAR has continued. Before we get to funding, why is this issue so personally important to you?

Q: Why is this a good investment for the American people and what are the prospects for continued U.S. leadership and funding?
(Set him up to show his funding slide)

For Favo:

Q: From your standpoint as a nurse who has worked with patients on the ground in Rwanda, to choosing to work at a systems-level to serve more people, what is happening today?

Q: Given the young people you've worked with in Rwanda, and you yourself as a young person, what's your take on feelings about prevention…and the state of the epidemic?

For Paul:

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Q: As an infectious disease doctor, you've been on the ground, working around the world in the most desperate areas. How has PEPFAR provided an infrastructure…and through the new paradigm we talked about earlier... a foundation to take on other diseases?

Q: From your perspective on the ground, what is your reaction to Dr. Fauci's comments on where we are with controlling the HIV pandemic and Favo's comments on the risk of HIV among young people?

For Favo:

Q: The effort to end the epidemic goes beyond traditional medical intervention. When you finish your Ph.D. at U Mass-Amherst, you plan to return to Rwanda and help educate nurses while also working in the community yourself. What will you tell your nursing students are the most important steps they can take - especially in terms of educating young people – to help end the epidemic?

Wrap-up question for each panelist

Q: As we've heard, there has been tremendous progress and there are many other problems in the world and here at home. From each of your perspectives, why should the American people still care?

What do you hope we'll see on the ground on the 25th anniversary of PEPFAR?

Paul?

Favo?

Tony?

Senator Graham, we'll give you the last word…

[8:00pm) Remain on stage as Ken Hersh comes up for closing comments]
8:05 p.m. Event Concludes

8:10 p.m. Program Participant Dinner
Presidential Reception Hall

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HHS-NIAID-20-0593-A-000734

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Tue, 8 May 2018 12:20:08 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Vance, Meaghan (NIH/NIAID) [C]
Subject: FW: May 9th event
Attachments: PEPFAR 2018 Key Messages.docx

These are from state dept since Birx cannot travel to (b)(6) Meaghan has added to your dallas folder but in case you want them electronically ...

Patricia L. Conrad
Special Assistant to the Director

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-----Original Message-----
From: Rancourt, Anne (NIH/NIAID) [E]
Sent: Wednesday, May 02, 2018 12:43 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6) Folkers, Greg (NIH/NIAID) [E]
(b)(6)
Cc: Billet, Courtney (NIH/NIAID) [E] (b)(6) Stover, Kathy (NIH/NIAID) [E]
(b)(6)
Subject: FW: May 9th event

Hi Patty and Greg,

Please see below/attached for PEPFAR talking points that they wanted to share with Dr. Fauci for his May 9 event. Amb. Birx cannot attend so if he can convey the accelerated impact of PEPFAR during the past four years as part of his comments, they would be appreciative.

Thanks,
Anne

On 5/2/18, 11:07 AM, (b)(6) wrote:

Hi Anne,

I know -- it's too bad the timing didn't work out.

Attached are some key messages we're using this year.

Thanks for passing them along.

Best,
(b)(6)

-----Original Message-----

AMERICAN OVERSIGHT

From: Rancourt, Anne (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, May 02, 2018 8:36 AM
To: Haroz, David
Subject: Re: May 9th event

David, thanks. Sorry to hear the AMB won't be there. If you have points ready to send, I'll happily pass them along to him. Thanks!

On 5/1/18, 7:16 PM (b)(6) wrote:

Hi Anne,

I hope you're well.

It's wonderful that Dr. Fauci is participating in the May 9th PEPFAR 15th anniversary event at the Bush Institute. Unfortunately, as you may know, Amb. Birx can't make it to the event as she'll be representing the USG at the Global Fund Board meeting in the Macedonia that week.

I'm sure it will happen organically, but it would be great if Dr. Fauci could convey some of the accelerated impact of PEPFAR during the past four years as part of his comments. If it would be helpful to send any brief related points, I'm happy to do so.

Thanks so much,
(b)(6)

Sent from my BlackBerry 10 smartphone.

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U.S. President's Emergency Plan for AIDS Relief (PEPFAR)

- PEPFAR was announced on January 28, 2003 and signed into law on May 27, 2003 and is up for reauthorization September 30, 2018. With strong bipartisan support across three administrations and from the Congress, the U.S. government has invested nearly $75 billion in the global HIV/AIDS response – the largest commitment made by any nation to address a single disease.

- This year, PEPFAR marks its 15-year anniversary and its remarkable efforts not only to transform the global HIV/AIDS response but also to save over 13 million lives. It also highlights the historic opportunity that, for the first time in modern history, we have the tools to control a pandemic without a vaccine or a cure, laying the groundwork for eventually eliminating HIV.

- PEPFAR has saved and improved millions of lives through its rapid acceleration of HIV prevention and treatment efforts, driven by transparent, accountable, efficient, and effective investments. PEPFAR is driven by data to achieve results – the U.S. government now supports lifesaving treatment for more than 13 million people globally, has enabled more than 2.2 million babies to be born HIV free, and assists more than 6.4 million orphans, vulnerable children, and their caregivers so they can thrive.

- The latest PEPFAR data also show a 25-40 percent decline or greater in new HIV diagnoses among adolescent girls and young women in the majority of the highest-HIV-burden communities implementing PEPFAR's DREAMS (Determined, Resilient, Empowered, AIDS-free, Mentored, and Safe) public-private partnership across 10 African countries.

- Under the leadership of the current U.S. Global AIDS Coordinator, Ambassador Deborah Birx, PEPFAR has increased the number of lives saved by 50% since 2014 within a budget-neutral environment by employing a robust data-driven and strategic business model approach.

- PEPFAR is a smart investment. The program is widely viewed as one of the most effective and efficient U.S. foreign assistance programs in history, using the latest data and science to direct resources where the HIV/AIDS epidemic is the largest, the need is highest, and dollars are most efficiently used.

- PEPFAR is accelerating its impact. Due to American leadership and the generosity of the American people, up to 13 high-HIV-burden countries are poised to achieve epidemic control by 2020 with PEPFAR support. These efforts will create the roadmap to reach epidemic control in the more than 50 countries that PEPFAR supports.

- Together, we are closer than ever to ending AIDS and decreasing the future costs of addressing the pandemic because of effective and focused programming. What once seemed impossible is now possible through the power of partnerships. PEPFAR proudly collaborates with partner governments, the private sector, philanthropic organizations, multilateral institutions, civil society and faith-based organizations, people living with HIV, and many others.

- PEPFAR has enhanced global health security, accelerating the progress toward a world more secure from infectious diseases threats by strengthening global capacity to prevent, detect, and respond to new and existing risks. PEPFAR investments in countries with sizable HIV/AIDS burdens have bolstered their ability to swiftly address Ebola, avian flu, cholera, and other outbreaks, which ultimately protects America's borders.

- The U.S. is advancing global health by working with partners to help countries reach development goals that advance common interests and values, reinforces stability in key regions, boosts U.S. economic opportunities, and establishes conditions for a more secure and prosperous world.

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From: Infectious Diseases-2018
Sent: Tue, 8 May 2018 09:27:20 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Dr. Anthony S. Fauci We appreciate your research work article

Dear Dr. Anthony S. Fauci,

Recognizing your busy schedule we would like to remind you regarding our upcoming meeting **International Conference on Infectious Diseases** conference in **Berlin, Germany** from **November 5-7, 2018.**

We read with great interest one of your article titled "**The Pathway to a Universal Influenza Vaccine**" which made us captivated with your scientific soundness.

Considering the scope of your article, we thought that you might be interested in presenting your latest work (oral/poster) at this meeting.

Infectious Diseases-2018 https://www.unitedscientificgroup.com/conferences/infectious-diseases/

It will be a very a good platform for researchers like you to showcase your work, to network with fellow attendees and get exposed to new ideas.

Please, let us know if this is something that would be of interest to you. We will send you more information about the event once we know your interest and availability.

Thank you for your time, hope to see you there soon!

Best Regards,

Tejasree
Conference Secretary
Infectious Diseases
Berlin, Germany
November 5-7, 2018
Email: (b)(6)
P: 408-426-4832/33

PS: If you find this mail unsolicited, please click here and type your email to unsubscribe https://unitedscientificgroup.com/unsubscribe.php and we will take care that you do not receive any further promotional mail.

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From: Folkers, Greg (NIH/NIAID) [E]
Sent: Tue, 8 May 2018 00:10:03 +0000
To: Conrad, Patricia (NIH/NIAID) [E];Fauci, Anthony (NIH/NIAID) [E]
Subject: PEPFAR 2018 report to Congress
Attachments: PEPFAR 2017 ANNUAL REPOR.pdf

The executive summary is ~28 pages and might be useful for Dallas folder

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PEPFAR

2018 ANNUAL REPORT TO CONGRESS

15 YEARS OF SAVING LIVES THROUGH
AMERICAN GENEROSITY AND PARTNERSHIP

AMERICAN OVERSIGHT

THE UNITED STATES PRESIDENT'S EMERGENCY PLAN FOR AIDS RELIEF

15 Years of Bipartisan Leadership

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Cover: A mother and her child attend a seminar on treatment adherence in Uganda. CREDIT: USAID

Left: President George W. Bush, President Barack H. Obama, President Donald J. Trump. CREDIT: The White House

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EXECUTIVE SUMMARY

With American leadership, we are at an unprecedented moment in the global HIV/AIDS response. For the first time in modern history, we have the opportunity to control a pandemic without a vaccine or a cure. We have the tools to do this, but the key will be comprehensive implementation brought to scale to reach people already living with HIV and prevent new infections. Controlling the HIV/AIDS pandemic will lay the groundwork for eventually eliminating or eradicating HIV, which will be possible through continued and future scientific breakthroughs in vaccine development and cure research.

Fifteen years ago, when the United States (U.S.) President's Emergency Plan for AIDS Relief (PEPFAR) was launched, these possibilities were unimaginable. Today, with the continued bipartisan support of the U.S. Congress and the leadership of President Donald Trump, the U.S. government is unquestionably the world's leader in responding to the global HIV/AIDS crisis.

Through the work of PEPFAR, in partnership with other governments and communities, the U.S. has saved millions of lives, prevented millions of new infections, and changed the very course of the pandemic. Since our inception in 2003, PEPFAR has received strong bipartisan support in Congress and through three administrations. The program has been reauthorized twice, with significant majorities. Conceived as a compassionate effort to save the lives of people in countries hardest hit by HIV/AIDS through the delivery of urgently needed treatment and care, PEPFAR is firmly poised to control and to ultimately end the pandemic as a public health threat. PEPFAR is demonstrating that resources can be laser-focused with clear strategies, constant assessment, and rapid programmatic corrections to increase their impact and achieve what was once an unimaginable goal.

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President Bush with a group of children during a visit to Africa.

CREDIT: George W. Bush Presidential Center

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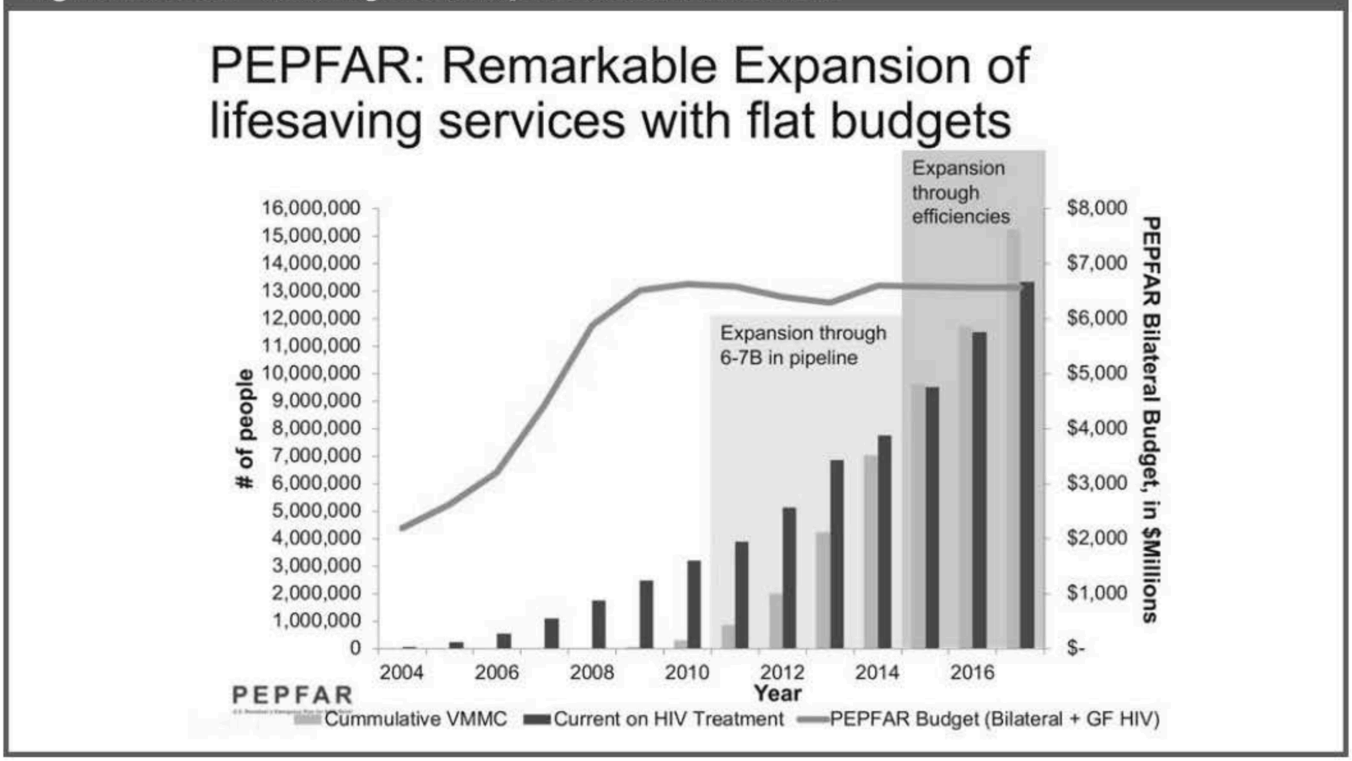
When signing PEPFAR into law in May 2003, through the U.S. Leadership Against HIV/AIDS, Tuberculosis and Malaria Act of 2003 (U.S. Leadership Act), President George W. Bush remarked that "*HIV/AIDS is one of the great medical challenges of our time...Across Africa, this disease is filling graveyards and creating orphans and leaving millions in a desperate fight for their own lives. They will not fight alone... The legislation I sign today launches an emergency effort that will provide $15 billion over the next five years to fight AIDS abroad... In the face of preventable death and suffering, we have a moral duty to act, and we are acting.*"

PEPFAR is widely regarded as one of the most effective and efficient foreign assistance programs in history, because our approach has shifted and our impact has expanded based on the latest science and the best available data. The result is enormous cost-efficiency – ensuring we have the greatest possible impact with each dollar entrusted to us by the American people. Over the past year, we have eliminated duplication, streamlined business practices, and shifted resources to serve those most in need and implement the most impactful interventions.

Our relentless commitment to accountability, transparency, and partnership has allowed the program to dramatically expand our results and impact in recent years without increases in budget (Figure A). PEPFAR continues to realign and refocus the program in every country where we work, employing better data and business practices to save more lives, prevent more infections, and accelerate progress toward controlling the epidemic. These critical program and business process improvements include targeting our investment using granular, site-level data; rigorous partner management to increase performance and efficiency; and intensive quarterly monitoring of the entire program. These efforts make PEPFAR a model for cost-effective foreign assistance.

Figure A: PEPFAR – Achieving Greater Impact on Current Investments



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A young girl in Uganda.

Furthermore, through the PEPFAR platform, the U.S. has enhanced global health security, accelerating the progress toward a world more secure from infectious disease threats. We have demonstrably strengthened the global capacity to prevent, detect, and respond to new and existing risks. PEPFAR's investments in countries with sizable HIV/AIDS burdens have bolstered their ability to swiftly address Ebola, avian flu, cholera, and other outbreaks, which ultimately protects America's borders. PEPFAR has an impact beyond HIV/AIDS and continues to invest strategically in the systems critical to ensure global health security. These invaluable lessons and experiences will continue to inform and improve the U.S. government's response to unforeseen health crises.

Above all, PEPFAR is an expression of the compassion and generosity of the American people. We are the iconic brand of U.S. government engagement in health, development, security, and diplomacy, unparalleled in our capacity to deliver clear, measurable, and transformative impact.

Spotlight

Increasing Our Impact Through Data-Driven, Cost-Effective Investments

PEPFAR is widely regarded as one of the most effective and efficient foreign assistance programs in history, because our approach has shifted and our impact has expanded based on the latest science and the best available data. As a result, PEPFAR's data-driven, cost-effective investments are accelerating progress toward controlling and ultimately ending the HIV/AIDS pandemic as a public health threat.

We work relentlessly to ensure we are delivering the greatest possible impact with every dollar that we invest. At every level of the program, we have eliminated duplication, streamlined business practices, and shifted resources to where they will be used in the most cost-effective manner to serve those most in need. This rigor has allowed PEPFAR to significantly expand our results and impact without increased financial resources.

We continue to realign and refocus the program in every country where we work, employing better data and business practices to save more lives, prevent more infections, and have the largest impact on the epidemic.

In advance of World AIDS Day 2017, President Donald Trump announced that PEPFAR has reached historic highs in our impact – preventing more HIV infections and saving more lives than ever before, driven by transparent, accountable, and cost-effective investments (Figure B).

As of September 30, 2017, PEPFAR had supported more than 13.3 million men, women, and children on lifesaving antiretroviral treatment (ART). When PEPFAR began in 2003, only 50,000 people were receiving ART in sub-Saharan Africa at

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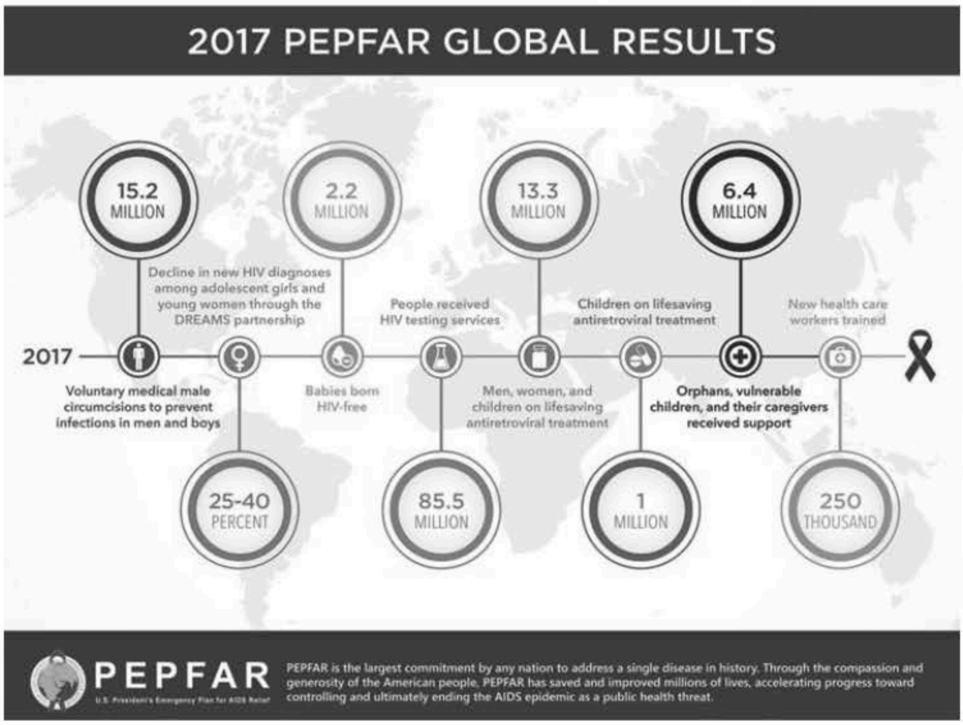
Figure B: PEPFAR – Latest Global Results, 2017



that time; communities were decimated with children left alone without mothers or fathers.

Today, we are saving lives not only by treating HIV, but also by stopping transmission before it happens, through three key prevention interventions. PEPFAR continues to be the driving force in preventing mother-to-child transmission. To date, PEPFAR has enabled more than 2.2 million babies to be born HIV-negative to HIV-positive mothers. To prevent new infections in boys and men, PEPFAR has supported more than 15.2 million of them through the provision of voluntary medical male circumcision (VMMC), including through the largest single-year increase (nearly 3.5 million) in VMMC results during fiscal year (FY) 2017, since the beginning of PEPFAR. To prevent infection in girls and young women, PEPFAR designed the groundbreaking DREAMS (Determined, Resilient, Empowered, AIDS-free, Mentored, and Safe) public-private partnership (PPP).

The latest PEPFAR data also show that, for the first time, we drove significant declines in new HIV diagnoses among adolescent girls and young women. In the 10 African countries implementing PEPFAR's pioneering DREAMS PPP, nearly two-thirds of the highest HIV-burden communities or districts achieved a 25–40 percent or greater decline in new HIV diagnoses among young women since 2015. Importantly, new diagnoses declined in nearly all DREAMS intervention districts. The DREAMS partnership is not only preventing HIV among adolescent girls and young women, but also empowering them to be the leaders of tomorrow.

To ensure children thrive and are protected from HIV, PEPFAR now supports more than 6.4 million orphans and vulnerable children (OVC) and their caregivers affected by HIV/AIDS. To ensure that children with HIV thrive, PEPFAR continues to focus more than $450 million annually on the diagnosis and treatment of children living with

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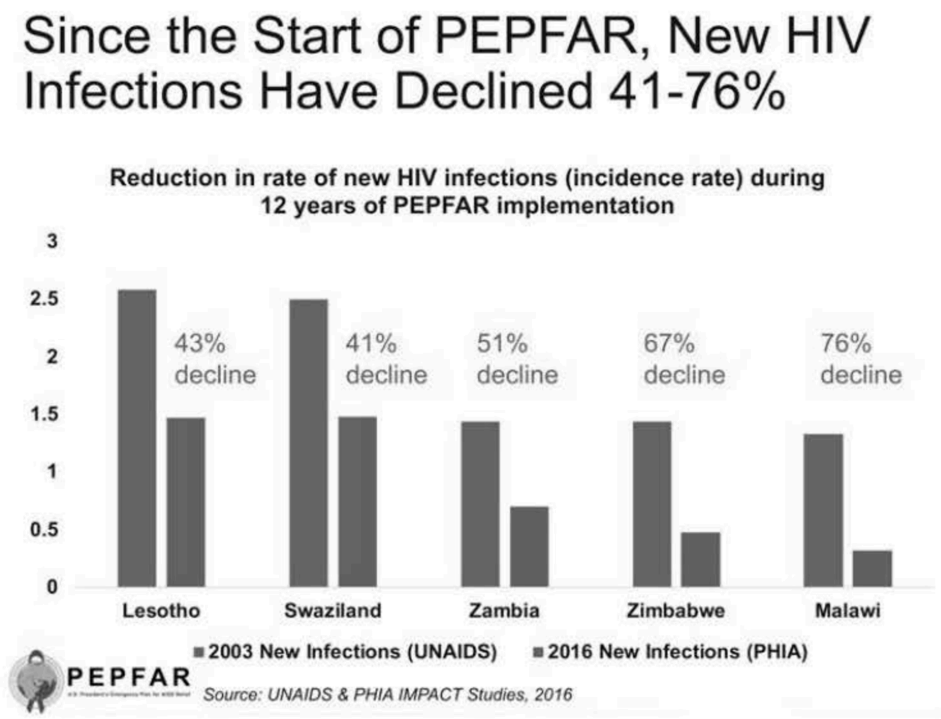
HIV, reaching more babies and children with lifesaving ART. And to ensure that more people can know their HIV status, PEPFAR supported more than 85.5 million people with HIV testing services in FY 2017 alone. All of these remarkable successes are possible due to our aggressive program monitoring focused on improving the impact of our investments quarter by quarter.

These new achievements add to PEPFAR's impact results released in the past year, which show that five African countries – Lesotho, Malawi, Swaziland, Zambia, and Zimbabwe – are approaching control of their HIV epidemics (Figures C and D). Epidemic control is the point at which new HIV infections fall below the total number of deaths from all causes among HIV-infected individuals. These countries are closing in on the targets set by the Joint United Nations Programme on HIV/AIDS (UNAIDS) as part of its 90-90-90 by 2020 goals – whereby 90 percent of people living with HIV (PLHIV) know their status, 90 percent of people who know their status are accessing treatment, and 90 percent of people on treatment have suppressed viral loads.[1] This fast-track strategy focused on controlling the HIV/AIDS pandemic has been endorsed by all of the countries where PEPFAR works and aims to ensure that 73 percent of all HIV-positive communities are virally suppressed, both for their own health and to reduce the possibility of their transmitting HIV.

These accomplishments also highlight critical advances being made under the *PEPFAR Strategy for Accelerating HIV/AIDS Epidemic Control (2017-2020)*. The Strategy was launched at the 2017 United Nations General Assembly (UNGA) and reaffirms the U.S. government's HIV/AIDS leadership and commitment in more

Figure C: New Infections Have Declined 43-76% Since the Start of PEPFAR



[1] UNAIDS. (2014). 90-90-90: An Ambitious Treatment Target to Help End the AIDS Epidemic. Retrieved from http://www.unaids.org/sites/default/files/media_asset/90-90-90_en_0.pdf

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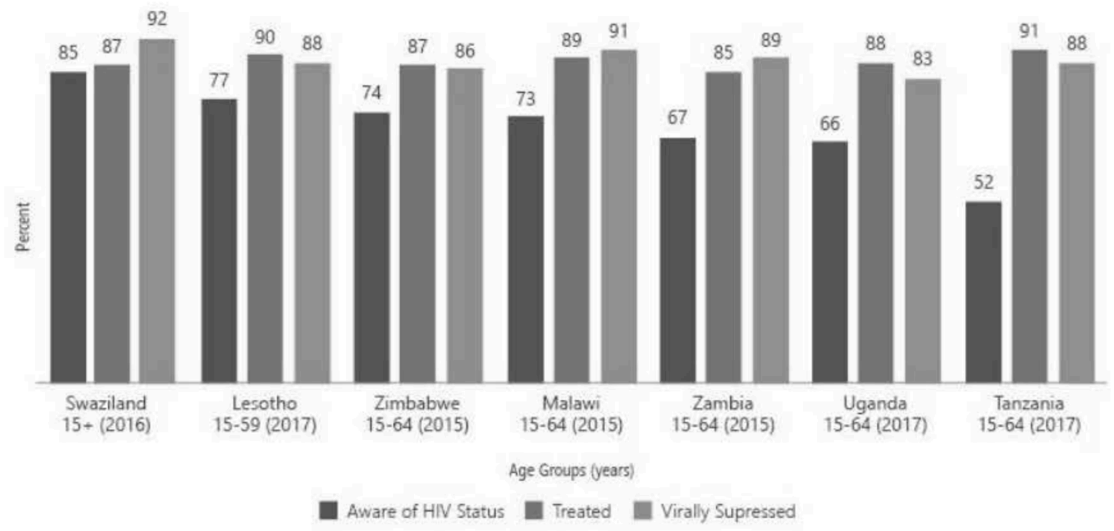
Figure D: Progress to 90-90-90 in Adults



than 50 countries. As the secretary stated in his introduction to the Strategy: "*The Trump administration remains deeply committed to the global HIV/AIDS response and to demonstrating clear outcomes and impact for every U.S. dollar spent. We want to look back together and recognize that it was here, at this point in time, that our accelerated focus led to a world free of HIV/AIDS.*"

At UNGA 72 in September 2017, President Trump underscored his support to PEPFAR, noting the program's overall importance and citing it as an example of how the U.S. government is doing more by finding greater efficiencies and ensuring we drive forward with impact and clear value for each dollar invested. As the President stated: "*We have invested in better health and opportunity all over the world through programs like PEPFAR.*"

With continued aggressive focus, quarterly data analysis, and partner alignment for maximum impact, under our new Strategy, PEPFAR is poised to help control the epidemic in up to 13 countries by 2020. Beyond saving an untold number of lives, this will reduce the future costs required to sustain the HIV/AIDS response. In less than two decades of PEPFAR's commitment and funding, the pandemic has the potential to progress from crisis to control. This would create the road map and the global evidence base for how future deadly pandemics can be controlled, by utilizing the best interventions and constantly assessing progress.

PEPFAR has built and strengthened the capacity of country-led responses in both the government and civil society while bringing key multisectoral partners to the table. We have fostered collaboration across the whole of the U.S. government, partner governments, and global partners, including multilateral institutions, civil society, faith-based organizations (FBOs), the private sector, philanthropic organizations, and PLHIV.

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PEPFAR's impact extends well beyond the health sector. The program has helped advance economic development, particularly in sub-Saharan Africa, by assisting in stabilizing countries and communities, creating returns on investment that will continue to pay dividends across multiple sectors long into the future. PEPFAR powerfully and unequivocally proves that investing in health is not only the right thing to do, but also the smart thing to do. PEPFAR has paved the pathway for effective and impactful foreign assistance by holding to the principles of accountability, transparency, and impact. We know that with continued focus we will control this epidemic.

The World Before PEPFAR

Before PEPFAR, controlling the HIV/AIDS pandemic was unimaginable. Reports from the front lines of the epidemic, especially in sub-Saharan Africa, were dire. In many countries, an HIV diagnosis was a death sentence, and entire families and communities were falling ill. Gains in global health and development were being lost. In the hardest-hit regions of sub-Saharan Africa, infant mortality doubled, child mortality tripled, and life expectancy dropped by 20 years. The rate of new HIV infections in the highest burden regions was exploding, and people were getting sick and dying during the most productive years of their lives. Millions of babies were becoming HIV-infected and millions of children were being orphaned by AIDS, leaving them susceptible to recruitment by negative influencers.

Despite early efforts to provide HIV prevention, care, and treatment services, at that time the epidemic continued to rage unabated. Lifesaving

Meet Hadijah – Empowering Girls to Fulfill Their Dreams

Outreach to adolescent girls and young women.

When Hadijah, a young woman in Uganda, became pregnant with her first child, she dropped out of school. Shortly afterward, her mother died, and she went to live with her father and then her stepfather, both of whom abused her, and she frequently went without shelter or food. Unfortunately, Hadijah also ended up living with an abusive boyfriend. Eventually the mother of four children, she began engaging in transactional sex to provide for her growing family, which exposed her to many risks, including gender-based violence.

It was Hadijah's love of sports that provided her the spark for positive change. One day at a netball meeting, a friend told her about a PEPFAR-supported program that helps adolescent girls and young women stay Determined, Resilient, Empowered, AIDS-free, Mentored, and Safe (DREAMS).

She enrolled, accessed HIV testing, and learned that she was HIV-negative. Within three weeks, she was equipped with DREAMS training on parenting and HIV prevention, and qualified as a peer facilitator. Thanks to DREAMS-supported hairstyling workshops, Hadijah now earns money braiding the hair of her netball teammates. Today, she is a local DREAMS safe space sports coordinator, netball team captain, and girl mentor. In addition, she runs a catering business for students at the DREAMS safe space.

"I am grateful for the knowledge I have acquired about HIV prevention, and I will continue to help other girls to make the right choices for their bright futures," Hadijah said.

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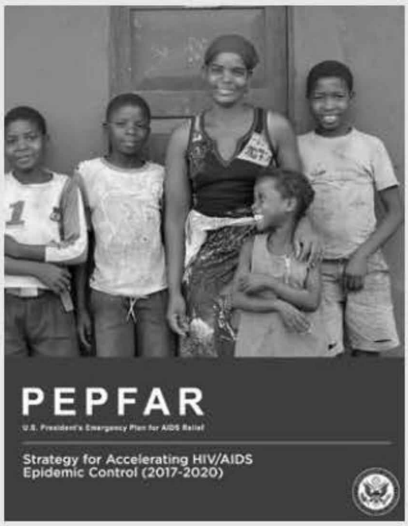
Spotlight



Under the PEPFAR Strategy for Accelerating HIV/AIDS Epidemic Control (2017-2020), which was launched at the 2017 United Nations General Assembly, PEPFAR is rapidly advancing efforts toward reaching epidemic control in more than 50 countries. With continued aggressive focus, quarterly data analysis, and partner alignment for maximum impact, under this Strategy, PEPFAR is poised to help control the epidemic in up to 13 countries by 2020.

We have already seen tremendous results. PEPFAR's Population-based HIV Impact Assessment data released in the past year show that five African countries – Lesotho, Malawi, Swaziland, Zambia, and Zimbabwe – are approaching control of their HIV epidemics. Epidemic control is the point at which new HIV infections fall below the total number of deaths from all causes among HIV-infected individuals.

Beyond saving an untold numbers of lives, achieving epidemic control will reduce the future costs required to sustain the HIV/AIDS response. In less than two decades of commitment and funding, the pandemic has the potential to progress from crisis to control. This would create the road map and the global evidence base for how future deadly pandemics can be controlled utilizing the best interventions and by constantly assessing progress.

medications that could turn the tide were inaccessible and unaffordable to virtually all but the richest. Further, many experts assumed that PLHIV in many parts of the world would be unable to sustain the complicated dosing regimens required for effective treatment.

The U.S. government responded. The U.S. Congress passed PEPFAR with strong bipartisan support just four months after President George W. Bush's call to action in his 2003 State of the Union address. With this unprecedented investment, the bright light of hope began to shine across the most devastated regions of the world.

Since our inception, PEPFAR has evolved through three major phases, all of which were underpinned by the significant leadership and support of the U.S. Congress.

PEPFAR I

During our first phase, under the leadership of President George W. Bush, PEPFAR was aptly named. The global HIV/AIDS epidemic was an emergency. By 2003, more than 20 million men, women, and children had died of AIDS-related illnesses in sub-Saharan Africa alone. Mothers, fathers, teachers, doctors, nurses, police, and soldiers were dying, and in the wake of their untimely deaths, 14 million children were left behind, without parents or communities to support them. There were substantial concerns, including those voiced by the U.S. National Intelligence Council, that HIV/AIDS was not only an unprecedented health crisis, but also a threat to security. In this phase, PEPFAR helped to reverse the death and devastation wrought by the epidemic, dramatically expanding access to HIV prevention, treatment, and care services – often where few if any such resources were previously available.

PEPFAR II

During our second phase, under the leadership of President Barack Obama, PEPFAR continued to expand access to HIV prevention and treatment,

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reaching millions more men, women, and children in need. The program also embraced a greater focus on country ownership and sustainability, seeking to ensure that the gains being made and the health delivery systems that were built to support them would be in place for the long term. In this phase, a range of new scientific breakthroughs – including VMMC, treatment as prevention, and more efficacious and less costly treatment regimens – were rapidly translated into program implementation, saving and improving more lives.

PEPFAR III

During our third (and current) phase, under the leadership of President Donald Trump, PEPFAR is firmly focused on controlling the HIV/AIDS epidemic as a public health threat. Building from the strong foundation from the prior years, PEPFAR is using the latest scientific tools and the best available data to rapidly accelerate progress toward epidemic control, driven by transparent, accountable, and cost-effective investments. With a strong commitment to advancing HIV/AIDS efforts in the more than 50 countries that PEPFAR supports, the program is also working to seize the unique opportunity to achieve epidemic control in up to 13 high-HIV-burden countries by 2020, creating the road map to reach epidemic control in all PEPFAR-supported countries. PEPFAR is also strengthening our partnerships with the private sector, faith-based community, and others that can help us to reach these goals.

Accelerating Progress Toward Epidemic Control

Today, thanks to PEPFAR and our partners, despair and death have been overwhelmingly replaced by hope and life. The HIV/AIDS

The Trump administration commemorates World AIDS Day by placing an AIDS Ribbon in front of the White House on December 1, 2017.

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A mother and her child visit a health clinic in Honduras.

epidemic is not only becoming controlled in five African countries, but the *PEPFAR Strategy for Accelerating HIV/AIDS Epidemic Control* (2017-2020) reaffirms the U.S. government's commitment to contributing to accelerated progress toward acheiving epidemic control in more than 50 countries. It also highlights the unique possibility that up to 13 high-HIV-burden countries are poised to control their epidemics by 2020 with continued focus and support. This bold Strategy, along with increased funding from implementing countries, provides a road map to sustain gains made from the U.S. government investment. Ending the health security threat posed by the epidemic is now possible, but it will not happen easily or automatically. We have the ability to finish what PEPFAR started, but it will take urgency, action, and focus. PEPFAR has made the impossible possible, and we are well positioned to do it again.

The American people – as they so often do – are leading the way. We can make our resources even more effective with respect to lives saved and infections averted as high-HIV/AIDS-burden countries expand treatment eligibility to all PLHIV, while at the same time, appropriately and aggressively implementing key recommendations for models of differentiated service delivery. Above all else, we must continue using data to drive decisions.

Delivering on the Vision

To accelerate progress toward controlling the epidemic and, ultimately, ending the AIDS pandemic as a public health threat, we must sustain and expand our collective gains. Led by our strategic vision, PEPFAR 3.0—Controlling the Epidemic: Delivering on the Promise of an AIDS-free Generation, we are using data to do the right things, in the right places, in the right way, and right now. PEPFAR 3.0 outlines how we can reach sustainable control of the

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HIV/AIDS epidemic through transparency, accountability, and impact. To deliver on this vision, PEPFAR is focused on four core priorities.

1. Increasing the Impact and Cost-Effectiveness of Every Dollar Invested

PEPFAR's data-driven, cost-effective investments are accelerating progress toward controlling and ultimately ending the HIV/AIDS pandemic. We use the latest science and the best available data to ensure we are delivering the greatest possible impact with every dollar that we invest. At every level of the program, we have eliminated duplication, streamlined business practices, and shifted resources to where they will be used in the most cost-effective manner to serve those most in need. This rigor has allowed PEPFAR to significantly expand our results and impact without increased financial resources.

PEPFAR works tirelessly to analyze data down to the site level to focus programs in the geographic areas and populations with the greatest HIV/AIDS burden. Data on HIV incidence, viral suppression, prevalence, and other key elements are essential to evaluating progress toward achieving epidemic control. PEPFAR disaggregates all of our data by sex, age, and geography in order to target and tailor our efforts to reach the specific needs of those we serve. These data inputs not only give us the clearest picture of the epidemic, but also our teams and partners the ability to respond efficiently to in-country challenges.

The PEPFAR-supported Population-based HIV Impact Assessments (PHIAs) provide valuable data on the progress we have made and the critical gaps that remain toward achieving HIV/AIDS epidemic control. To date, seven countries have completed PHIAs (six additional countries will conduct PHIAs on a rolling basis from 2018–2019). These assessments show that five of these countries – Lesotho, Malawi, Swaziland, Zambia, and Zimbabwe – are approaching control of their HIV/AIDS epidemics. In Swaziland, for example, HIV incidence dramatically declined by more than 45 percent between 2011 and 2016, coinciding with the U.S.'s increased investment in

Spotlight

A group of young children in South Africa. CREDIT: USAID

Ensuring Children Survive and Thrive

PEPFAR is the world's largest supporter of children living with and affected by HIV/AIDS.

PEPFAR continues to contribute 10 percent of all of our program funds to mitigate the physical, emotional, and economic impacts of HIV/AIDS on children. To ensure children thrive and are protected from HIV, as of September 30, 2017, PEPFAR had supported more than 6.4 million orphans and vulnerable children and their caregivers affected by HIV/AIDS.

To ensure that children with HIV thrive, PEPFAR continues to focus nearly a half billion dollars annually on expanding the diagnosis and treatment of children living with HIV. This includes by identifying and addressing key barriers to diagnosing children living with HIV and by working with industry to ensure that more child-friendly, efficacious, and affordable antiretroviral treatment regimens are produced. Without treatment, 50 percent of HIV-positive children will die before their second birthday, and 80 percent before turning 5 years of age. As of September 30, 2017, PEPFAR had supported nearly 1 million children on lifesaving ART.

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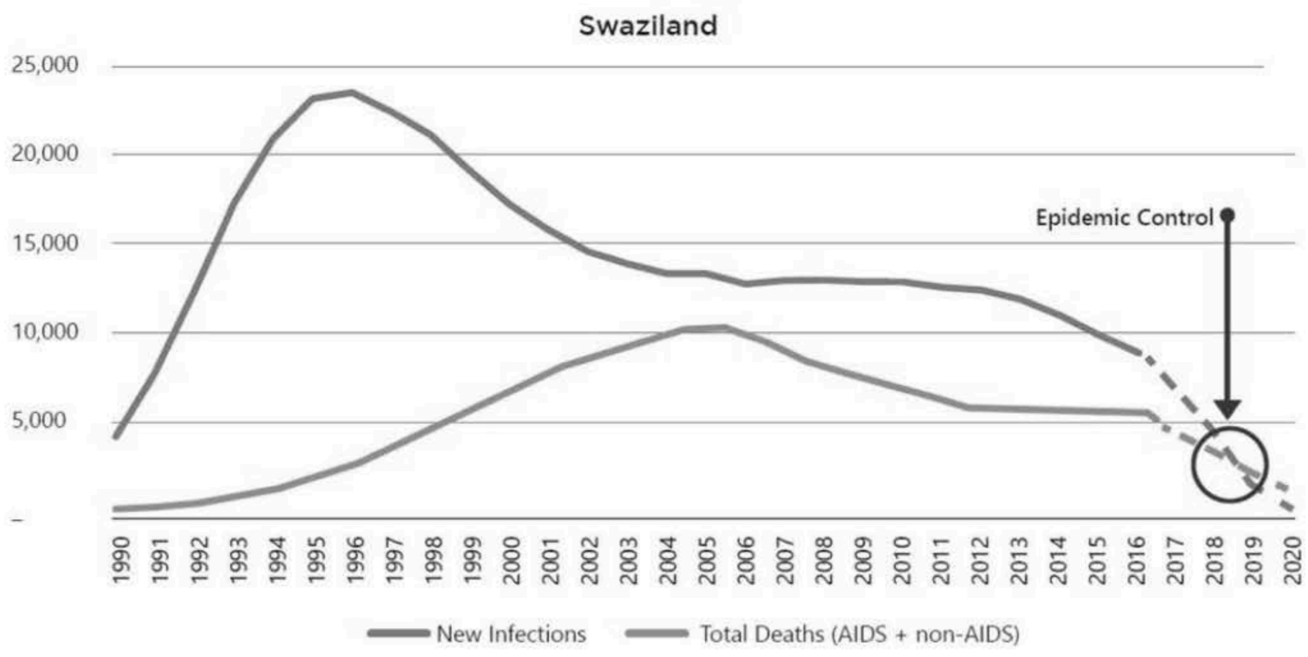
Figure E: Swaziland – Pathway to Reaching Epidemic Control



and focus on expansion of effective programming in partnership with the Kingdom of Swaziland. The repeat PEPFAR-supported HIV incidence and prevalence survey showed that the country is projected to achieve epidemic control if we maintain our momentum and focus (Figure E).

Across these countries, the major drivers of success were data-driven strategies to increase HIV treatment coverage; expand access to proven HIV prevention interventions, such as VMMC; and reduce new HIV infections among adolescent girls and young women through the PEPFAR-led DREAMS partnership. In the two countries where the PHIA results show that progress was slower – Tanzania and Uganda – these strategies were not nearly as focused and the coverage of core interventions is lower, but has the ability to expand rapidly and increase impact. A recent study in Uganda's Rakai District published in the New England Journal of Medicine shows that progress is possible and the lessons from Rakai must be utilized throughout Uganda to increase impact and achieve epidemic control.

While the PHIA results demonstrate tremendous gains, they also reveal key gaps in HIV prevention and treatment programming for children, younger men and younger women that require urgent attention and action. Children under 15 years of age have inadequate access to HIV diagnosis and treatment. PEPFAR is committed to improve service access for babies and children by analyzing data and holding ourselves accountable. In all seven completed PHIAs, young women and men under age 35 were far less likely to know their HIV status, be on HIV treatment, or be virally suppressed than older adults. These gaps are all areas in which PEPFAR continues to invest and innovate under our new Strategy. In particular, PEPFAR will continue to advance efforts to reduce HIV incidence among adolescent girls and young women through the DREAMS PPP and reach and link more young men to HIV services.

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Creating Male-Friendly HIV Services

Data from many countries show that men, especially those ages 20–35, tend to access HIV testing at lower rates than do young women. As a result, men living with HIV often enter care with more advanced disease, are less likely to receive lifesaving antiretroviral treatment, miss opportunities to prevent ongoing transmission, and have higher AIDS-related mortality than their female counterparts.

To help break down barriers to men accessing HIV prevention and treatment, the PEPFAR-supported Witkoppen's Mvuselelo Male-Friendly Clinic in South Africa has created an environment where men can relax and address their health problems. Witkoppen, a winner of the PEPFAR-funded DREAMS Innovation Challenge, consulted directly with men in developing the clinic. Male patients identified barriers such as long wait times, and the importance of male staff and flexible operating hours. "Mvuselelo provides a space where we can talk freely without fear of being stigmatized," says one patient.

Outreach to young men.

The clinic is a one-stop shop for men, offering primary health services such as screening and management of sexually transmitted infections and other acute and chronic conditions; HIV testing, counseling, care, and treatment; and health education, as well as referrals for other health needs. Many men who visit Mvuselelo also have learned to discuss HIV transmission risk reduction, treatment, and care with their partners.

Through the PEPFAR Dashboards, we make PEPFAR data public to drive greater impact, transparency, and accountability for all stakeholders, including U.S. citizens, communities around the world, civil society organizations, U.S. government agencies, donors, and partner country governments. Anyone, anywhere may view and utilize PEPFAR-planned funding, program results, and expenditure analysis data in an accessible and easy-to-use format. By using data, we have improved partner performance and increased program efficiency and effectiveness. As part of this effort, the Interagency Collaborative for Program Improvement (ICPI) brings together experts from PEPFAR's seven implementing agencies to analyze, monitor, and optimally allocate the resources needed to control the epidemic.

We also rigorously manage our partners to increase their performance and efficiency and conduct intensive quarterly reporting and monitoring of the entire program. In the end, we are all accountable to the U.S. Congress and the American people, who put their trust in PEPFAR to save lives and to adapt and evolve in order to deliver the greatest possible return on investment. The people we serve and the American taxpayer deserve nothing less.

2. Focusing Prevention for Impact

PEPFAR's prevention investments are having their greatest impact in the history of the program.

As of September 30, 2017, PEPFAR had supported more than 15.2 million men and boys in eastern

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DREAMS Partnership

The latest PEPFAR data show that, for the first time, we have driven significant declines in new HIV diagnoses among adolescent girls and young women. In the 10 African countries implementing PEPFAR's pioneering DREAMS (Determined, Resilient, Empowered, AIDS-free, Mentored, and Safe) public-private partnership, nearly two-thirds of the highest HIV-burden communities or districts have achieved a 25–40 percent or greater decline in new HIV diagnoses among young women since 2015. Importantly, new diagnoses have declined in nearly all DREAMS intervention districts. The DREAMS partnership is not only preventing HIV among adolescent girls and young women, but also empowering them to be the leaders of tomorrow.

Alongside DREAMS, we have invested substantially to find and reach more men with HIV prevention and treatment services, including through applying innovative approaches to bring men into the health care system, which they often otherwise do not access until they are ill.

and southern Africa with substantial protection from HIV infection through the provision of VMMC. In FY 2017 alone, PEPFAR supported the largest single-year increase (nearly 3.5 million) in VMMC procedures since the program's inception.

Every year, 360,000 adolescent girls and young women are infected with HIV – nearly 1,000 a day. Gender-based violence (GBV) is a significant reason why, affecting millions of adolescent girls and young women. Girls who experience violence are up to three times more likely to be infected with HIV or other sexually transmitted infections. Violence Against Children (VAC) surveys (most of them supported by PEPFAR) in 11 countries found that an average of one in three children reported their first sexual experience was forced or coerced. PEPFAR will need to move from the primary focus on post-rape care to a laser focus on the prevention of rape of girls ages 9–14 to help ensure all children can thrive, HIV-free.

To address these alarming statistics, PEPFAR launched the DREAMS PPP on World AIDS Day 2014. On World AIDS Day 2017, we reported that in the 10 African countries implementing DREAMS programs, nearly two-thirds (65 percent) of the highest HIV-burden communities or districts have achieved a 25–40 percent or greater decline in new HIV diagnoses among young women since 2015. Importantly, new diagnoses declined in nearly all of the 63 DREAMS intervention districts. DREAMS private sector partners include the Bill & Melinda Gates Foundation, Gilead Sciences, Girl Effect, Johnson & Johnson, and ViiV Healthcare.

To ensure the sustainability of our DREAMS efforts, PEPFAR integrated DREAMS programming and budgets into our 2017 Country Operational Plans (COP 2017). In COP 2017, we also added DREAMS-like programming in five additional countries – Botswana, Côte d'Ivoire, Haiti, Namibia, and Rwanda – with a particular focus on girls ages 9–14 to prevent sexual violence and prevent HIV through avoiding sexual risk.

Also, on January 23, 2017, President Trump issued a Presidential Memorandum reinstating the January 22, 2001 Presidential Memorandum on the "Mexico City Policy" for the U.S. Agency for International Development's (USAID) family planning assistance and directing the secretary

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Beneficiaries of PEPFAR's PMTCT programs in Swaziland.

of state, in coordination with the secretary of Health and Human Services, to implement a plan to extend the Mexico City Policy to "global health assistance furnished by all departments or agencies" to the extent allowable by law. The expanded policy is referred to as Protecting Life in Global Health Assistance (PLGHA). PEPFAR works with the relevant U.S. government agencies to implement the policy consistently, examine progress toward its implementation, and monitor its effects to ensure U.S. taxpayer money is not used to fund foreign organizations that perform or actively promote abortion as a method of family planning in other nations.

PEPFAR continues to contribute 10 percent of all of our program funds to mitigate the physical, emotional, and economic impacts of HIV/AIDS on children. As of September 30, 2017, PEPFAR had provided care and support to more than 6.4 million orphans, vulnerable children, and their caregivers. We have also enhanced the quality of services across our OVC programs to support even better health outcomes for children. Additionally, PEPFAR is collaborating with UNAIDS and other partners on the *Start Free Stay Free AIDS Free* framework for children, adolescents, and young adults.

We cannot end the HIV/AIDS epidemic through medical interventions alone. We must also address the underlying social issues, especially unequal human rights and stigma and discrimination, that prevent people from accessing the medical interventions of HIV prevention and treatment. When any person is stigmatized or unable to access services due to discrimination, the health of everyone in the community is threatened and the epidemic continues to expand rather than contract.

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U.S. Secretary of State Rex Tillerson shakes hands with Mozambique President Filipe Jacinto Nyusi before their bilateral meeting at the U.S. Department of State in Washington, D.C., on June 14, 2017.

PEPFAR has specific initiatives addressing the dynamics driving stigma, discrimination, and violence as a part of our broader efforts to expand key populations' access to and retention in quality HIV/AIDS prevention and treatment services.[2] Programs such as the Key Populations Investment Fund, the Key Populations Implementation Science Initiative, and the Local Capacity Initiative work to understand, document, and respond to the unique needs of these populations, as well as strengthen the implementation capacity of key population-led and other civil society organizations. PEPFAR continues to prioritize efforts and leverage partnerships to ensure all individuals have safe, nondiscriminatory access to HIV prevention and treatment. But we need to move to more precise measurements of stigma and discrimination to ensure we are having a clear impact and that service access is improving.

PEPFAR partners with the Elton John AIDS Foundation on a $10 million Key Population Fund initiative to strengthen organizations working to meet the HIV needs of Lesbian, Gay, Bisexual, and Transgender (LGBT) people, with a focus on sub-Saharan Africa and the Caribbean. PEPFAR is also a founding donor of the Robert Carr Civil Society Networks Fund, which supports civil society networks to support inadequately served populations' access to HIV services. Through PEPFAR's various collaborations, such as with the Elton John AIDS Foundation and the Robert Carr Civil Society Networks Fund, and our own initiatives, such as the Key Populations Investment Fund, the program is investing in civil society leadership and capacity to increase quality access to HIV services for key populations.

3. Accelerating Access to HIV Treatment

As of September 30, 2017, PEPFAR had supported more than 13.3 million men, women, and children on lifesaving HIV treatment – nearly twice as many as only four years ago.

HIV treatment is one of the most cost-effective investments toward controlling the epidemic, both for the health of the patient receiving the medication and to prevent onward transmission of HIV. Science shows that one of the most important factors in the successful treatment of HIV is the early initiation of ART. The sooner that a person living with HIV begins treatment, the more intact their immune system remains and the faster they can achieve viral suppression, which virtually eliminates their risk of them transmitting the virus.

[2] UNAIDS. (2014). 90-90-90: An Ambitious Treatment Target to Help End the AIDS Epidemic. Retrieved from http://www.unaids.org/sites/default/files/media_asset/90-90-90_en_0.pdf

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PEPFAR has worked aggressively with our partner countries to adopt and rapidly implement "Test and Start" approaches to HIV treatment, whereby an individual who tests positive for HIV is immediately offered lifelong ART. By combining this approach with the implementation of other key policies priorities – such as using differentiated models of care, including multi-month ART prescriptions for stable HIV patients and same-day initiation of ART for new patients – we can dramatically increase the coverage of ART, improve retention in HIV treatment programs, and reduce the financial and time burden placed on both national health systems and our patients. With these critical changes, we can treat more people with the same financial resources.

We are also committed to using the most efficacious, safest, quality-assured, and lowest cost ART regimens possible. For this reason, PEPFAR is working with partner governments and multilateral partners to support the rapid adoption of tenofovir/lamivudine/dolutegravir (TLD) as a first-line ART for adults and adolescents currently receiving efavirenz-based first-line ART or for those who are ready to start ART. This will not only be better for the patient, but also it will reduce overall costs of PEPFAR support into the future.

PEPFAR is the world's largest supporter of children living with and affected by HIV/AIDS. Without treatment, 50 percent of HIV-positive children will die before their second birthday, and 80 percent before turning 5 years of age. PEPFAR is significantly expanding access to pediatric treatment. This includes by identifying and addressing key barriers to diagnosing children living with HIV and by working with industry to ensure that more child-friendly, efficacious, and affordable ART regimens are produced. As of September 30, 2017, PEPFAR had supported nearly 1 million children on lifesaving ART and continues to invest nearly a half billion dollars annually to ensure children are prioritized within PEPFAR.

Spotlight

Religious leaders gather with school children to commemorate the Day of the African Child on June 16, 2017.

CREDIT: World Council of Churches

Strengthening Our Partnership with Faith-Based Organizations

From the earliest days of the epidemic, faith-based organizations (FBOs) have been central to the HIV/AIDS response. They remain indispensable partners in the effort. In many PEPFAR countries, FBOs are the largest nongovernmental provider of HIV services. This is why PEPFAR has committed more than $1 billion in planned funding to more than 40 faith-based partners since 2012.

PEPFAR continues to strengthen our partnership with FBOs. One of the most important facets of our commitment to work with faith-based partners is our shared focus on pediatric treatment and our ambitious goals toward ending AIDS in children, adolescents, and young women by 2020 as captured by the *Start Free Stay Free AIDS Free Framework*.

In November 2017, PEPFAR, along with the Vatican Dicastery for the Promotion of Integral Human Development, UNAIDS, Caritas International, Ecumenical Advocacy Alliance – World Council of Churches, the WHO, and the Elizabeth Glaser Pediatric AIDS Foundation, convened leaders of major pharmaceutical and medical technology companies, multilateral organizations, donors, governments, faith-based partners, and other key stakeholders for a High-Level Discussion on Scaling Up Early Diagnosis and Treatment of Children and Adolescents. Participants gathered to discuss ways to expedite the research, development, approval, introduction, and uptake of optimal drugs and formulations for infants, children, and adolescents.

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To expand access to HIV treatment, PEPFAR continues to work closely with The Global Fund to Fight AIDS, Tuberculosis and Malaria (Global Fund), partner country governments, and others, with a focus on reducing duplication and maximizing impact – so that the most people in need of ART can be reached with all available resources.

There are also numerous economic benefits from treating PLHIV before they develop AIDS. Healthy HIV-infected individuals on treatment are able to work and support their families. Keeping parents healthy also lessens other social costs, such as caring for children whose parents die of AIDS-related illnesses. Robust statistical models show the economic benefits of treatment will likely exceed program costs within just a decade of investment. In other words, treating people not only saves lives, but also generates considerable economic returns.

Serving Children in Kenya

Mercy Millicent only learned her own HIV status after her mother died in 2007. Her aunt took her to Lea Toto for treatment, and there Mercy found much more than just medication. She learned to accept her status, and participated in Lea Toto programs around life skills, education, and counseling. Today, she is 22 and a student at the Kenya Institute of Management. Mercy credits Lea Toto for her thriving. "Today, I am who I am because of Lea Toto. Acceptance came from within."

Lea Toto means "to raise a child" in Swahili, and the work done by the Children of God Relief Institute (COGRI) in Kenya at their Lea Toto Program has helped to raise more than 10,000 HIV-positive children to lead their healthiest and fullest lives. Lea Toto is just one of the four essential programs run by COGRI in Kenya; there is also Nyumbani Home, Nyumbani Village, and Nyumbani Lab.

Lea Toto aims to improve the quality of life of HIV-positive children living in eight of Nairobi's poorest informal settlements. Since its founding in 1999, it has become one of the largest nongovernmental pediatric HIV/AIDS providers in Kenya. It currently cares for more than 3,000 HIV-positive children who live with a family member, and facilitates a holistic process to health care that involves comprehensive medical care, provision of antiretroviral drugs, nutrition and food services, psychosocial support, education, and vocational training.

Sister Mary with a group of children at Lea Toto in Kenya.

CREDIT: COGRI

Additionally, Lea Toto is at the forefront of adolescent outreach, and the program focuses on teaching comprehensive life skills and economic empowerment, while building capacity.

PEPFAR and USAID have been longtime funders of the work done by COGRI; for example, PEPFAR funds 80 percent of the Lea Toto program and provides all antiretroviral treatment to the children living at Nyumbani Home. Sister Mary Owens, COGRI's executive director, says that the impact of these essential funds is clear when you look around and see their children grow up. "It gives us the chance to do more work, and also a means of creating awareness among people and properly informing them about what HIV is: It is simply a medical condition that can be contained, and young people living with it can still live full lives."

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4. Leveraging Partnerships for Sustainability

No single actor alone can control and ultimately end the HIV/AIDS pandemic. We need all sectors and diverse partners working together to provide financing, demonstrate political will, and carry out interventions both within and outside of the health sector, and we must always include people directly affected by HIV in any response.

PEPFAR works closely with our partner countries toward achieving epidemic control while promoting the long-term sustainability of their HIV/AIDS responses. We use the new tools we have developed, including the Sustainability Index and Dashboard, to track progress on our overall goals, as outlined in the Sustainable HIV Epidemic Control PEPFAR Position Paper. We must continue to act decisively and strategically with our resources and continue to bring other donors and partner countries to the table to respond to and ultimately help end the epidemic. Over the past few years under U.S. leadership, many countries have substantially increased their own investments in controlling HIV. Several countries in southern Africa provide the majority of available resources to address their own epidemics, including by covering the lion's share of cost for HIV treatment. These countries include Botswana, Namibia, South Africa, and Swaziland. Vietnam is also taking over the primary cost of HIV treatment. Each year, additional PEPFAR-supported countries are increasing their own investment in their response and critically focusing these investments on the most effective interventions, creating a partnership with impact.

Working with multilateral partners such as UNAIDS and the Global Fund, PEPFAR optimizes our investments, strengthens country leadership and sustainability, and enhances service delivery. UNAIDS is the international standard bearer in HIV/AIDS for setting critical goals in the global call to end the epidemic. Our collaboration with UNAIDS supports countries in overcoming key policy, programming, and implementation challenges. Over the last several years, PEPFAR and UNAIDS have worked together to build global commitment to achieve the UNAIDS 90-90-90 treatment goals as a key benchmark in reaching epidemic control and moving toward the end of the AIDS epidemic. The increased partnership between PEPFAR and the Global Fund, to which we are the largest donor, maximizes the impact of our joint investments through the more strategic use of resources to support programs that are impactful and sustainable. PEPFAR also dedicates initiatives to strengthen civil society engagement, leadership, and capacity, including with FBOs, recognizing that sustainable HIV/AIDS interventions must be tailored to and informed by the communities we serve.

A local pastor greets a child in the highlands of Papua New Guinea. The pastor teaches his congregation about HIV transmission and prevention.

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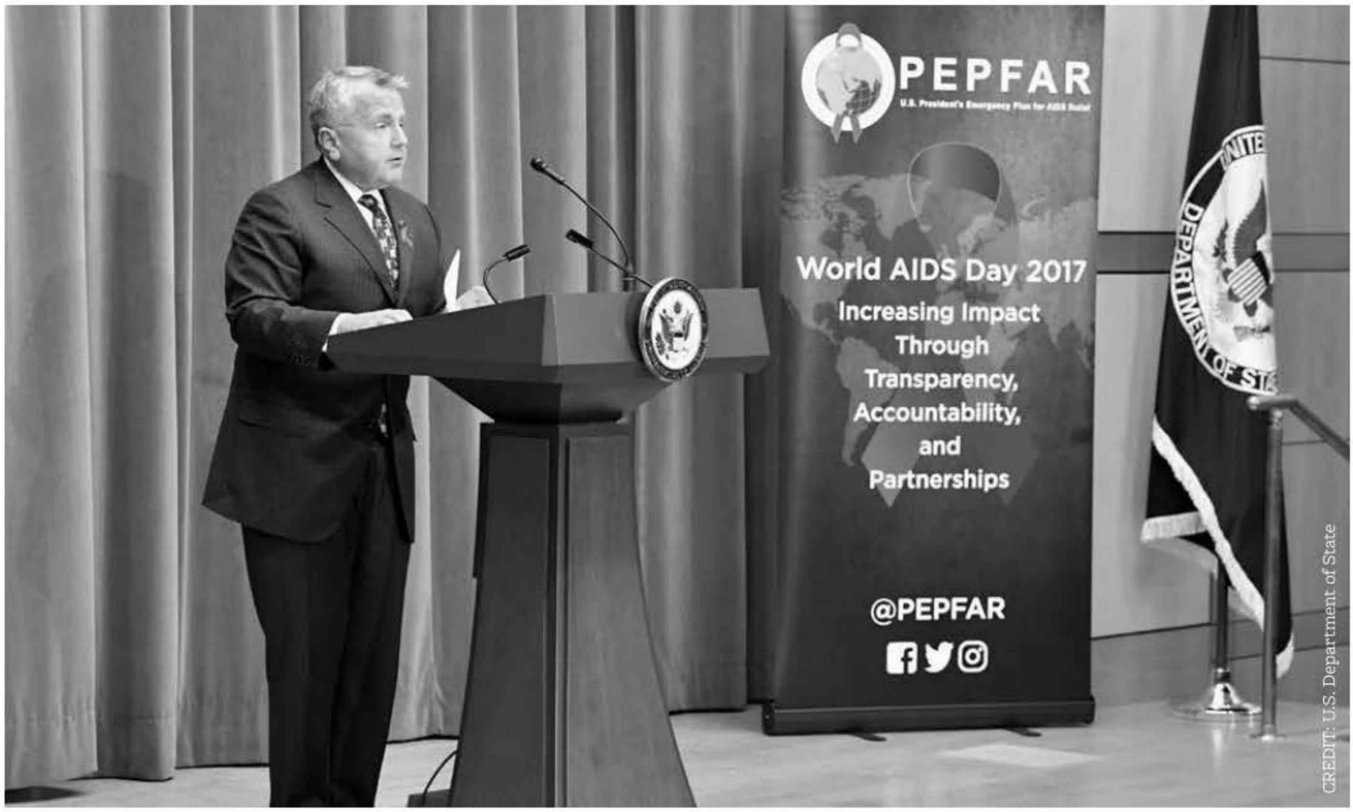

CREDIT: U.S. Department of State

Deputy Secretary of State John Sullivan delivers remarks to commemorate World AIDS Day at an event at the U.S. Department of State in Washington, D.C., on November 30, 2017.

PEPFAR forges strategic PPPs that support and complement our HIV prevention, care, and treatment work. These partnerships translate new ideas into practice and accelerate impact, benefiting from the private sector's expertise and resources as well as its leadership, energy, and inspiration. PEPFAR also advances global health diplomacy through close engagement with U.S. chiefs of mission in partner countries, as well as with their counterpart foreign diplomats based in Washington, D.C., to increase the impact of U.S. health investments and partnerships.

Since our founding, PEPFAR has built health infrastructure and strengthened capacity through an emphasis on sustainability. This infrastructure and capacity not only support people living with HIV/AIDS, but also are leveraged for maternal and child health, tuberculosis, malaria, immunizations, and emergency disease outbreak responses. We invest in robust laboratories and well-trained laboratory specialists critical to well-functioning health systems, enabling clinicians and health workers to better diagnose and treat a range of diseases and conditions. PEPFAR has trained nearly 250,000 health care workers to deliver and improve HIV care and other health services, creating a lasting infrastructure that enables partner countries to address all health challenges of today and tomorrow.

Key Remaining Challenges

We have a narrow window to change the course of the pandemic and put the world on track to end AIDS by 2030, the target set by the global community when 193 countries adopted the 2030 Agenda for Sustainable Development and the Sustainable Development Goals. We must seize this historic opportunity.

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According to UNAIDS, nearly 37 million people are living with HIV globally; however, the number of those on treatment is currently 20.9 million. While treatment access has increased by more than 160 percent since 2010, there is still much more to do to ensure everyone is virally suppressed, especially children and individuals under age 35. Constant evaluation of program implementation, epidemic data, and partner performance is essential to continue to accelerate our impact. We must continue to act decisively and strategically with our resources and to bring other donors and high-HIV-burden countries to the table; otherwise, we all risk an epidemic that rebounds beyond the global community's capacity to respond.

The time to act is now. Every week, 32,000 people are infected with HIV globally, including 6,900 young women and 3,000 children, and 19,000 people die of AIDS-related illnesses. In sub-Saharan Africa, adolescent girls and young women are especially affected. Of all the new HIV infections in adolescents in the region, nearly 75 percent are among females; they are up to 14 times more likely to contract HIV/AIDS than young men.

This is particularly concerning as the population of those ages 15–24 in sub-Saharan Africa is set to double in size by 2020, reaching 200 million (Figure F). This "youth bulge" comprises 100 million more young people in sub-Saharan Africa than we had in 1990. The result: millions more young people who are entering a time in life when they are most susceptible to HIV infection, often without an education or job opportunities. These demographic trends mean that we must work hard just to keep up, and we must work even harder and faster to stay ahead of the epidemic.

As results from the PHIAs demonstrate, while we see strong progress for older adults and children, there are critical gaps in HIV prevention and treatment programming for younger men

Figure F: Youth Bulge in Sub-Saharan Africa

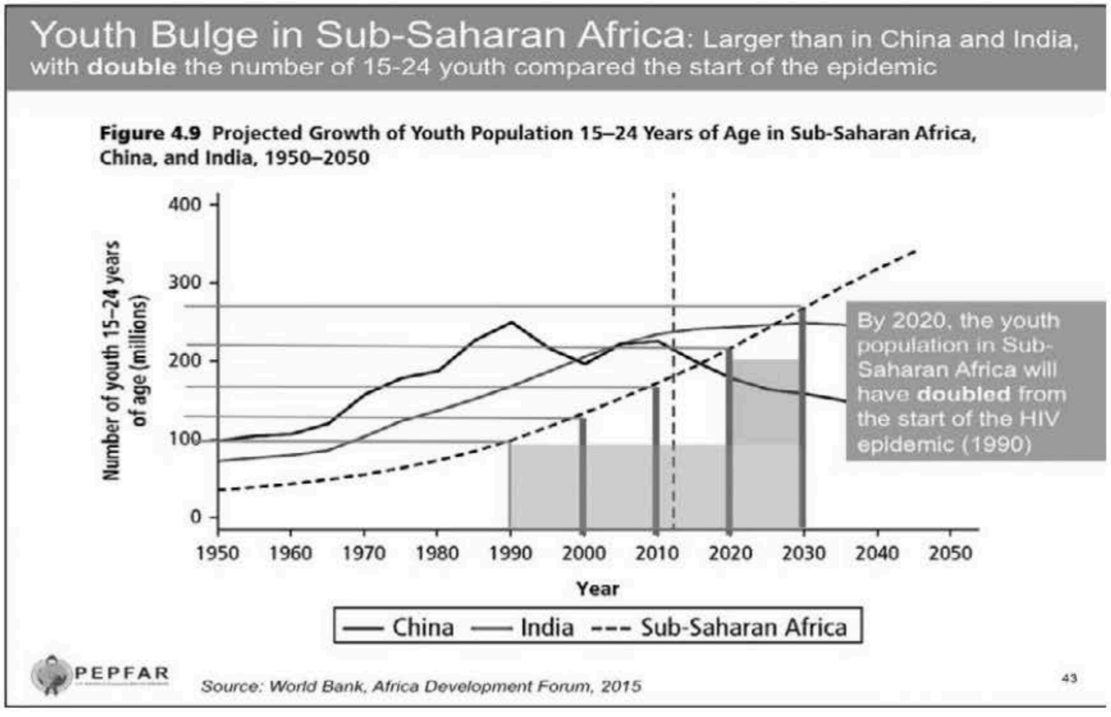

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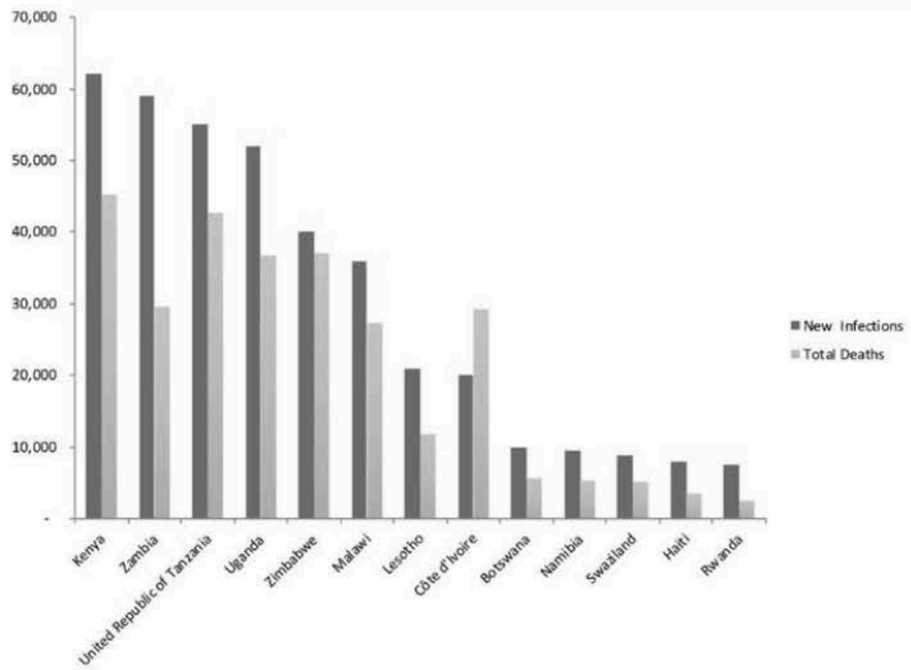
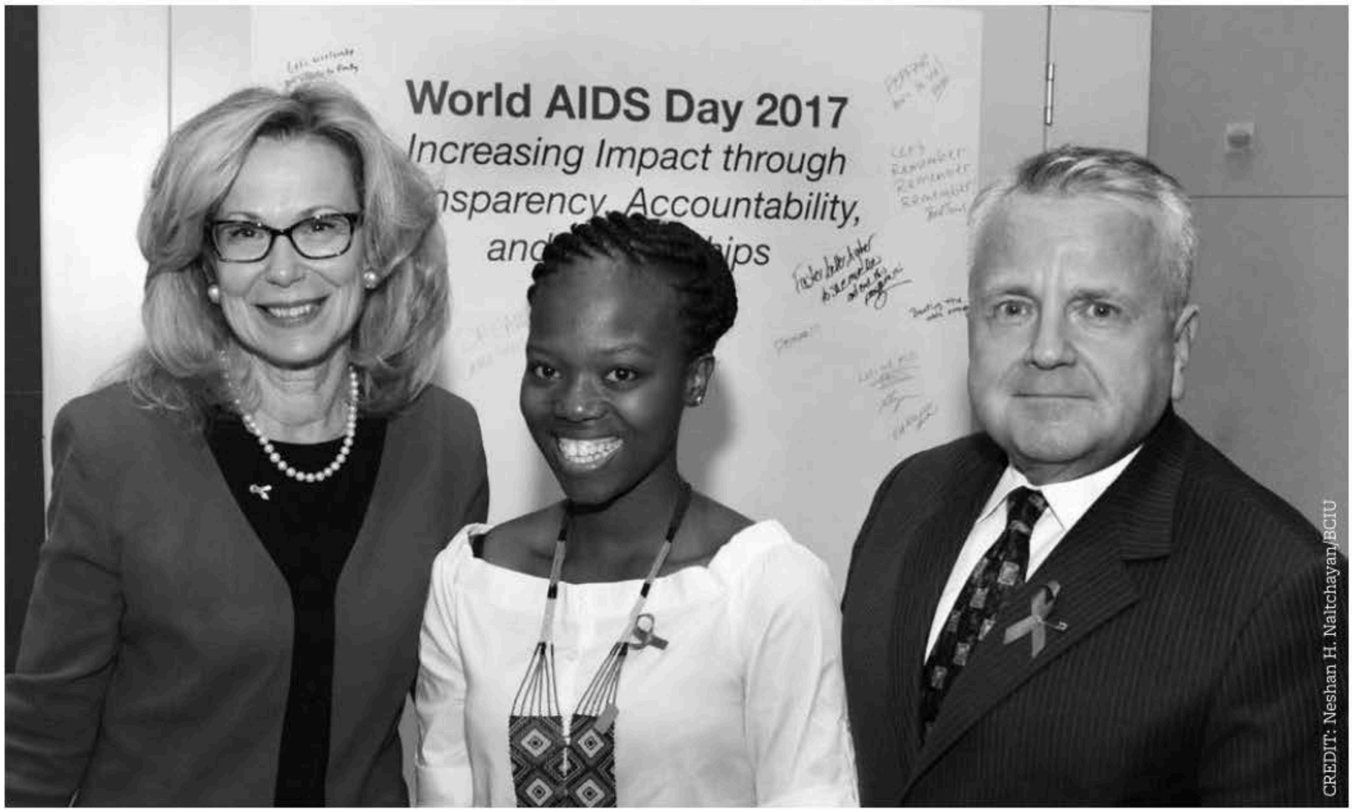
Figure G: Progress Needed to Achieve Epidemic Control in 13 High-Burden Countries





Deputy Secretary of State John Sullivan (right), celebrating World AIDS Day with DREAMS Ambassador Nontokozo Zakwe (middle) and Ambassador Deborah L. Birx (left) at the U.S. Diplomacy Center.

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and women that require urgent attention and action. In all seven surveys conducted to date, young women and men under age 35 were far less likely to know their HIV status, be on HIV treatment, or be virally suppressed than older adults. Despite important gains achieved through DREAMS, adolescent girls and young women remain highly vulnerable to infection. We are also still struggling to find and reach many younger men with HIV services. Moreover, key populations are too often relegated to the shadows by stigma, discrimination, and violence.

Above all, while the gains we have made together are remarkable, they are also fragile and can be quickly reversed if we slow down or grow complacent. Protecting our investment to date, ensuring control of this pandemic, and decreasing the future costs required to address HIV/AIDS are our daily focus and will be the key to our collective success.

PEPFAR Strategy for Accelerating Progress Toward Epidemic Control

The *PEPFAR Strategy for Accelerating HIV/AIDS Epidemic Control* (2017-2020) reaffirms the U.S. government's leadership and commitment, through PEPFAR, to accelerate efforts toward epidemic control in more than 50 countries, ensuring access to services by all populations, including the most vulnerable and at-risk groups. It also sets a bold course for accelerated PEPFAR implementation in a subset of 13 high-burden countries with the greatest potential to achieve HIV/AIDS epidemic control by 2020 (Figure G). PEPFAR will support these 13 countries to achieve at least 90 percent of PLHIV who know their status, 90 percent of people who know their status accessing treatment, and 90 percent of people on treatment having suppressed viral loads across all ages, genders, and at-risk groups by 2020. Epidemic control will only be attained when these targets are met for adults and children.

This Epidemic Control Strategy focuses and aligns U.S. government resources and activities by emphasizing the following five action steps:

- Acceleration of optimized HIV testing and treatment strategies, particularly to reach men under age 35. This is important, as we know that more than half of men under age 35 do not know their HIV status and are not on treatment, which is fueling the epidemic in young women ages 15–24 and young men ages 25–35

Spotlight

Targeting Public-Private Partnerships for Impact

Partnerships with the private sector play a critical role in advancing progress toward ending the HIV/AIDS epidemic. PEPFAR strategically focuses on our public-private partnerships (PPPs) to increase programmatic impact and efficiency. PEPFAR's PPP strategy includes finding opportunities where the private sector can complement PEPFAR goals and priorities by leveraging private sector brands, distribution networks, innovation, technology, and market-driven approaches to help achieve epidemic control.

Much like the private sector, PEPFAR is focused on accountability and scale. PEPFAR often looks to business models of private sector companies for ideas on how to run the program most effectively and efficiently. PPPs enable PEPFAR to not only share risks, resources, and rewards, but also find greater efficiencies in program delivery.

In 2017, PEPFAR developed, implemented, and sustained 16 global PPPs. In addition to achieving greater efficiencies within the program, these partnerships demonstrate PEPFAR's continued commitment to achieving epidemic control among children, adolescent girls and young women, boys and men, and key populations.

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A patient receives an HIV test at a mobile clinic in Thailand.

- Expansion of HIV prevention, particularly for young women under age 25 and men under age 30, through the scale-up of innovative and successful DREAMS efforts and the expansion of VMMC for boys and young men in targeted age bands
- Continuous use of granular epidemiologic and cost data to improve partner performance and increase program impact and effectiveness
- Renewed engagement with FBOs and the private sector to accelerate and improve efforts toward epidemic control and ensuring access to lifesaving services for children
- Strengthened policy and financial contributions by partner governments in the HIV/AIDS response

Conclusion

We have the historic opportunity to control and ultimately end the HIV/AIDS pandemic as a public health threat. This year marks the 15th anniversary of PEPFAR. Since our inception, PEPFAR has worked tirelessly to strengthen our partnerships, increase efficiencies, and expand impact, driven by a relentless commitment to transparency and accountability. Our stewardship over PEPFAR is inspired and enabled by the compassion and generosity of the American people and the U.S. Congress, which we honor daily through our commitment to programmatic excellence and oversight, and to the millions of men, women, and children whom we are privileged to serve around the world.

PEPFAR thrives due to the exceptional contributions from within the U.S. government.

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Around the globe, the U.S. ambassadors have been essential in ensuring that critical policy changes occur, so our dollars are utilized more effectively, and that our partnerships are more accountable and transparent. The leadership at the U.S. Department of State's Office of the Global AIDS Coordinator and Health Diplomacy, combined with the implementation through USAID; the U.S. Department of Health and Human Services and its agencies, including the Centers for Disease Control and Prevention, Health Resources and Services Administration, and the National Institutes of Health; the Department of Defense; the Peace Corps; and the Department of Labor, as well as our partnership with the Department of the Treasury, demonstrate the true strength of the whole-of-government approach. The dedicated career staff working internationally under the leadership of the chiefs of mission at our embassies overseas are vital to bilateral engagement and ensuring successful implementation.

Together, we apply a data-driven, targeted approach to address one of the most complex global health crises in modern history. The U.S. challenged the conventional wisdom that nothing could be done to reduce new HIV infections and control the epidemic in high-burden countries by dramatically expanding evidence-based, community-focused HIV prevention, treatment, and care programs in under-resourced settings. Time and again, PEPFAR has made the impossible possible.

Each day we wake up guided by the memory of the 35 million men, women, and children who have died from AIDS-related illnesses since the start of the epidemic. PEPFAR is determined to work even harder and smarter to save and improve the lives of the nearly 37 million people who are still living with the disease.

For the first time, the end of the HIV/AIDS epidemic is in sight. The U.S., through PEPFAR, continues to lead the way.

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INCREASING THE IMPACT AND COST-EFFECTIVENESS OF EVERY DOLLAR INVESTED

PEPFAR is committed to using data to focus investments in evidence-based interventions in the geographic areas and populations with the greatest HIV/AIDS burden for maximum impact. Utilizing data for decision-making is critical to reach those in most need of HIV services. Programmatic and surveillance data on HIV incidence, viral suppression, and prevalence across gender and all age groups are essential to evaluating progress toward the achievement of epidemic control.

PEPFAR disaggregates all of our data by sex, age, and geography in order to target and tailor our efforts to reach the specific and unique needs of those we serve. These data inputs give us not only the clearest picture of the epidemic, but also our teams and partners the ability to respond efficiently to in-country challenges.

We also rigorously manage our partners to increase their performance and efficiency, and conduct intensive quarterly reporting and monitoring of the entire program. In the end, we are all accountable to the United States (U.S.) Congress and the American people, who put their trust in PEPFAR to save lives and to adapt and evolve in order to deliver the greatest possible return on investment. The people we serve and the American taxpayer deserve nothing less.

Delivering more with every dollar means that PEPFAR will continue to use data and collaborate with partners to look for the best possible solutions to reach the most people in need of HIV/AIDS services with our available financial resources. The following section focuses on how PEPFAR uses data to monitor progress, identify and address key gaps, and document the incredible progress that has already been achieved.

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A group of school girls in Botswana.

CREDIT: USAID

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How PEPFAR Harnesses Data for Maximizing Cost Effectiveness and Impact: Controlling the HIV/AIDS Pandemic

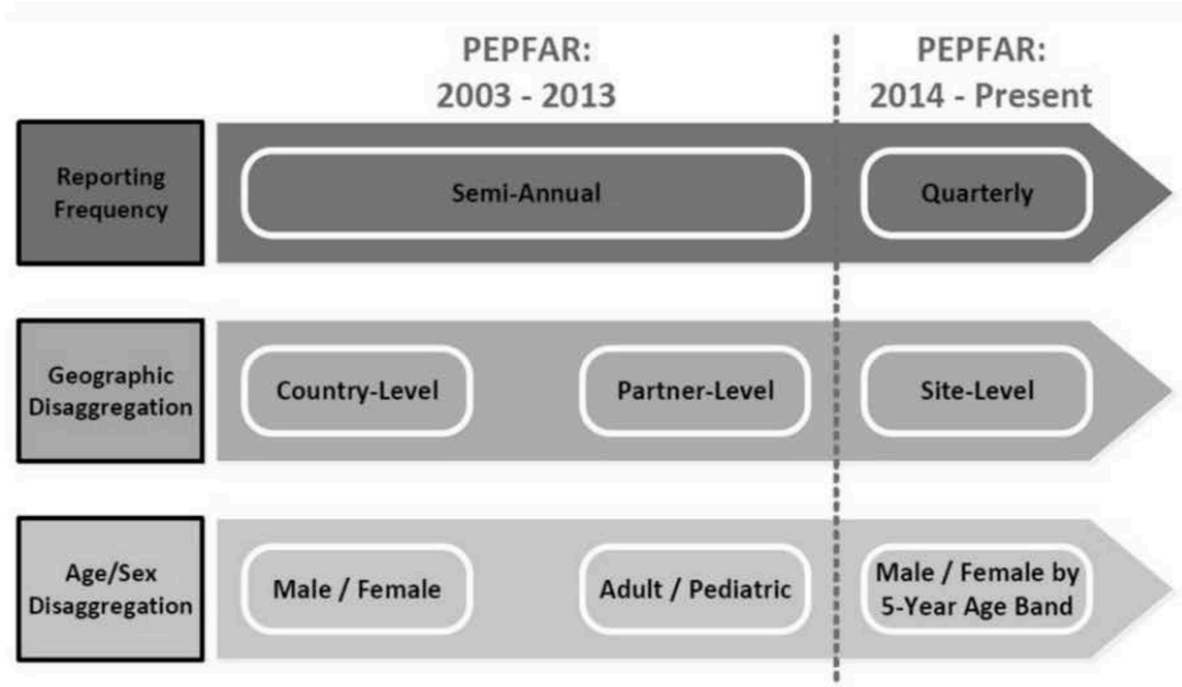
Since 2003, the U.S. President's Emergency Plan for AIDS Relief (PEPFAR) has continuously updated the way we program, collect, and use data to reach the front edge of the HIV/AIDS epidemic (Figure 1). In prior years, when data collection was less frequent or granular, countries relied heavily on survey results and modeling to assess their epidemics and underserved populations.

While nationally representative surveys like Demographic Health Surveys and previously conducted AIDS Impact Surveys are excellent sources of information, these large surveys are expensive and typically take a long time to complete and disseminate findings. Resulting delays in programmatic course corrections would allow an epidemic to continue to expand without targeted interventions.

Surveys such as Population-based HIV Impact Assessments (PHIAs) are critical to detect changes in incidence and prevalence by geography and age groups, and as high-prevalence countries are approaching the Joint United Nations Programme on HIV/AIDS (UNAIDS) 90-90-90 targets, it is critical to tailor programs to reach those populations and places with the highest HIV burden and risk. In order to tailor a country program appropriately between surveys, PEPFAR program data can be used as a proxy, especially as the use of new HIV incidence assays are taken to scale.

The 90-90-90 targets set by UNAIDS and endorsed by member countries aim to have 90 percent of all people living with HIV (PLHIV) to know their HIV status, 90 percent of all people with diagnosed HIV infection to

Figure 1: Then and Now: Innovations Maximize PEPFAR's Efficiency



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A group of young boys in Lesotho.

receive sustained antiretroviral therapy (ART), and 90 percent of all people receiving ART to have viral suppression by 2020. PEPFAR has expanded the definition of 90-90-90 to include all age groups and genders to ensure that everyone at risk is accessing prevention and treatment and that progress is evident.

The benefit of using PEPFAR data is that it is collected frequently and provides disaggregated data by age, gender, and geographic location. Additionally, site-level data collected by PEPFAR partners are owned by the country government and can be used and disseminated as needed. Quarterly reporting and review allow for real-time data use so public health program managers can more easily keep up with the epidemic.

Since PEPFAR began collecting data on key indicators at the site level and by age and sex, data quality has significantly improved the information available to inform critical programmatic shifts. One example of using PEPFAR data to stay on top of the epidemic can be seen in Swaziland. Prior to the PEPFAR-supported Swaziland HIV Incidence Measurement Surveys (SHIMS2) published in 2017, the last incidence survey in Swaziland had been completed in 2012. Incredibly, over five years, Swaziland reduced its HIV incidence by almost half. However, looking at the result of SHIMS2, the evidence of viral load suppression among young men ages 15–34 is significantly lower than that of young women the same age. These results are consistent with a population of young men who are HIV-positive and have either not been diagnosed or are not active and adherent on treatment. This is the same group that data show are most likely to transmit the virus, especially to younger women.

In Figures 2 and 3, PEPFAR data show a similar trend in examining ART coverage and viral

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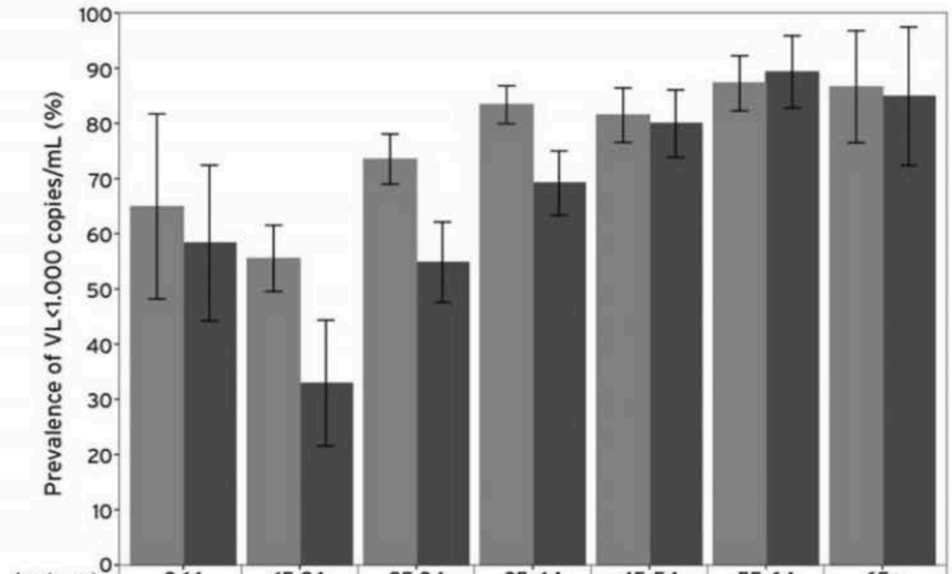
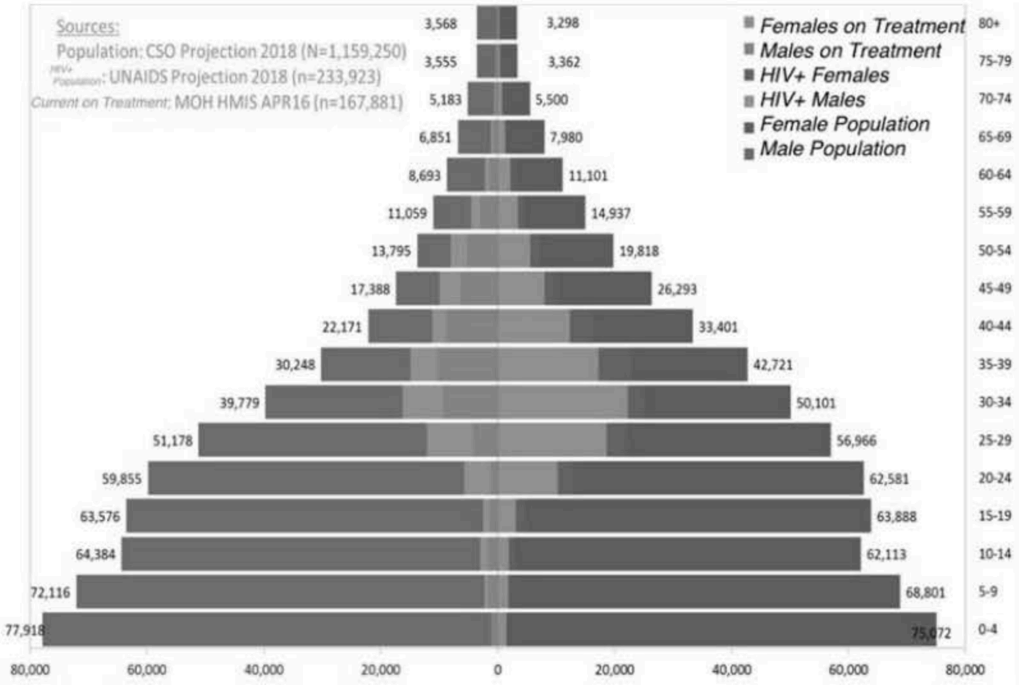
Figure 2: Swaziland SHIMS Prevalence of Viral Load Suppression, 2017



Age (years)	0-14	15-24	25-34	35-44	45-54	55-64	65+
Females	64.9%	55.5%	73.5%	83.4%	81.5%	87.3%	86.6%
Males	58.3%	32.9%	54.8%	69.2%	80.0%	89.3%	84.9%

Error bars represent 95% confidence intervals.

Figure 3: PEPFAR-Reported Swaziland Treatment Coverage



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load suppression by age and sex. The coverage among men ages 15–34 is considerably lower than among same-aged females. Using this PEPFAR-reported data, a country team could decide that young men are a critical intervention point and create new and innovative ways to reach this population for HIV diagnosis and treatment. Ensuring that all populations are effectively reached with prevention and treatment are key to ensuring our impact on the epidemic and the most lives saved. In many geographic areas, infants, children, and young men are especially underdiagnosed and treated. These analyses must be constant to ensure we stay ahead of the epidemic and ensure everyone at risk and in need of treatment is reached.

How PEPFAR Documents Results

PEPFAR's focus on optimizing outcomes and impact is a driving force behind global efforts to reach epidemic control. PEPFAR is partnering with the international community to accelerate toward reaching the UNAIDS 90-90-90 targets in all five-year age disaggregated populations to ultimately reach 95-95-95 at the country level by 2030. This translates to ensuring 95 percent of all PLHIV know their status, 95 percent of all people who know their HIV status are accessing treatment, and 95 percent of people on treatment have suppressed viral loads.

Within PEPFAR, teams assess populations and geographies, design interventions, and set targets aimed at accelerating epidemic control based on the clarity provided by the data. This allows the program, in partnership with governments and communities, to focus services, stop or improve the activities that are not having the desired outcomes, and expand those activities that are reaching essential groups. To enhance the systematic gathering, analysis,

Young children in Vietnam.

synthesis, and interpretation of program data for routinely measuring progress, PEPFAR has defined a core set of program indicators that are collected and reviewed at least quarterly.

Going forward, PEPFAR planning will be based on five-year age bands for 10- to 40-year-olds (Figure 4) as well as those at the most vulnerable ages, including under 1 and 1–9 years, to efficiently target and implement programs for specific populations as identified by the latest PHIA findings. Progress toward epidemic control will be successfully measured in part through an effective strategic information framework that monitors not only program outputs, but also key outcomes and programmatic impact.

In order to monitor progress in all populations, PEPFAR relies on the quarterly submission of data from all country teams. It is no longer adequate

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A man brings his wife to a men's health event for HIV testing in Malawi.

Figure 4: New Focused Age Bands for PEPFAR Programming

	5-Year Age Bands for 90-90-90 Attainment
Gender Independent	• <1 • 1-9
Male/ Female	• 10-14 • 15-19 • 20-24 • 25-29 • 30-34 • 35-39 • 40-49 • 50+

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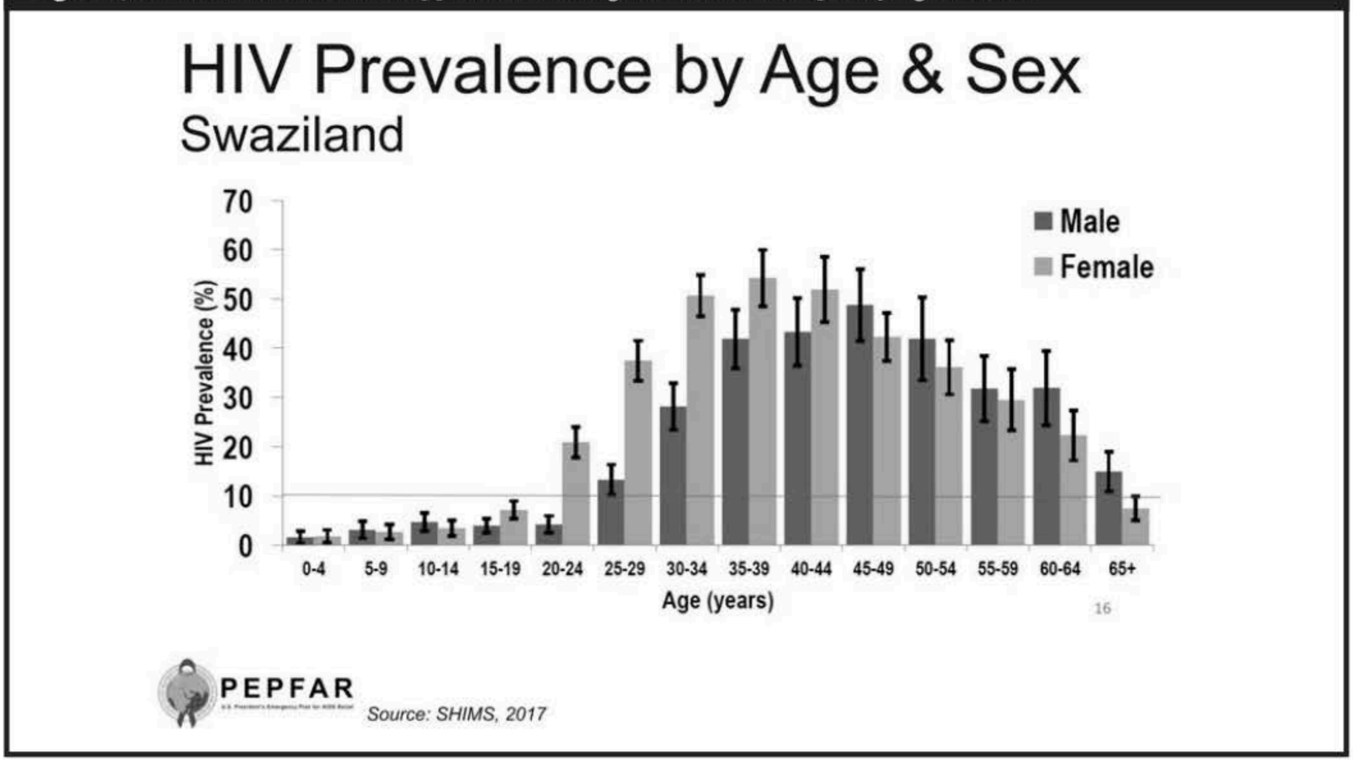
Figure 5: Swaziland Viral Load Suppression Among HIV-Positive People, by Age and Sex



to collect data at the aggregate level, as the needs of the individual people within the population differ among and even within the countries.

PEPFAR relies on our robust set of Monitoring, Evaluation, and Reporting (MER) indicators that collect site-level programmatic results by age, sex, and, in some cases, key population (KP) for each person receiving PEPFAR-supported services at a site. In the most recent version of the MER indicators (Version 2.2), there is an increased focus on understanding the nuances of how adult populations access services to reach treatment saturation and viral suppression. Whereas previously adults in the 25- to 49-year-old age band were targeted similarly, the recent PHIA results showed that adults in the 25- to 49-year-old age band access services differently.

For example, Figure 5 shows the prevalence of viral load suppression by age and sex in Swaziland based on SHIMS2 data. Prevalence of viral load suppression among men is not uniform among men older than 25. Specifically, men ages 25–34 and 35–44 had much lower rates of viral suppression than men in other age bands and women within the same age band. These findings emphasize the need to further disaggregate the data reported to PEPFAR in order to better understand the factors driving these differences.

To ensure PEPFAR is reaching the populations with the highest need, the MER Version 2.2 encourages data collection in five-year age bands from ages 0 to 50. Using these more granular age bands, public health program managers are better able to tailor programs to reach the appropriate populations with treatment and adherence services.

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A group of young boys celebrate during the Day of the African Child in Nairobi, Kenya, an event to highlight the rights of children and adolescents living with HIV.

Global Trends in New HIV Infections

PEPFAR supports strategic core HIV prevention and treatment interventions in order to ensure that the number of new HIV infections is lower than the number of total deaths among all HIV-positive individuals – an essential metric in reaching epidemic control. Figure 6 documents the tremendous reduction in the number of new HIV infections that has been achieved since the peak of the epidemic.

Particularly notable is the progress made in sub-Saharan Africa, where PEPFAR invests more than 90 percent of our Country/Regional Operational Plan (COP/ROP) resources. The only regions with an increase in new infections during this time period are Eastern Europe and Central Asia, where the numbers are primarily driven by an increase in new HIV infections in Russia, accounting for 80 percent of all new infections in the region. PEPFAR strategically invests in the Ukraine, which is responsible for 10 percent of all new infections in the region.

When the number of new infections is less than the number of total deaths among all HIV-positive individuals, the total burden of disease and the financial cost of the epidemic will decline community-by-community, county-by-county, and country-by-country. Importantly, these numbers need to be analyzed on a community-by-community and country-by-country basis to ensure success. The number of annual new infections across all PEPFAR-supported countries was 2.2 million in 2003, 1.6 million in 2013, and 1.3 million in 2016. Accelerating this downward trend, to reach under 1 million, is critical to

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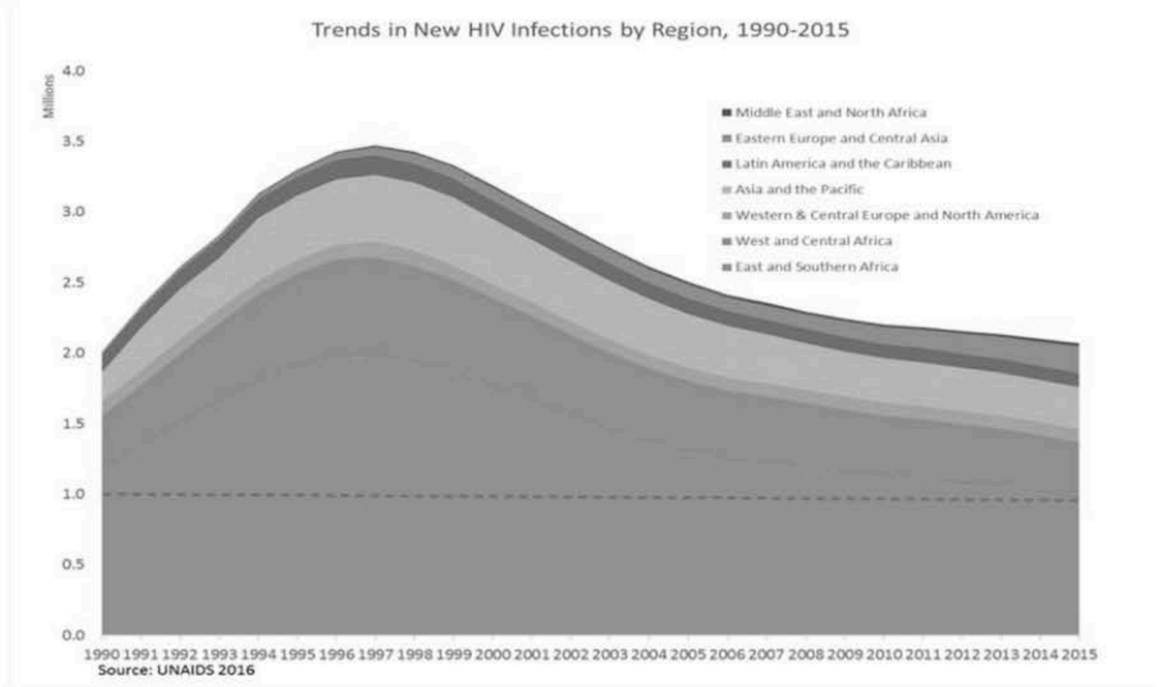
Figure 6: Global Trends in New HIV Infections, 1990–2015



achieving control of the pandemic across PEPFAR, geographic area by geographic area.

PEPFAR is focused on continuing to reduce new infections by saturating areas of high HIV burden at the most local subnational level (region, district, and subdistrict) with prevention and treatment services, including targeted HIV testing services (HTS). By strategically refocusing, PEPFAR programs will identify and treat many more HIV-infected persons, reducing new infections and lowering the average viral load in the high-transmission communities that we support. Tracking down to this granular level and with these details will be critical to success. Viral load is defined as the amount of HIV particles in a sample of blood, and individuals with high viral loads are more likely to transmit HIV to uninfected individuals. Those with undetectable viral loads are unable to transmit the virus – either between mothers and babies in utero and during breastfeeding, and sexually between couples.

Ensuring saturation with HIV prevention services in the same high-transmission zones will also have the greatest impact on the epidemic. These efforts will focus on increasing the coverage of evidence-based combination prevention interventions among the following priority populations: young men ensuring 100 percent circumcision rates to prevent young men from acquiring HIV; discordant couples; key populations; tuberculosis (TB)/HIV co-infected patients; children; pregnant and breastfeeding women; and young women and girls through Determined, Resilient, Empowered, AIDS-free, Mentored, and Safe (DREAMS) and orphans and vulnerable children (OVC) programming. In addition, program data has recently highlighted that we have historically underserved young men, who often go on to infect younger women, fueling the cycle of HIV infection. We launched special efforts to identify and treat HIV-positive men for 2016–2017 COPs/ROPs, and these underserved young men will be a continued area of focus for 2018 COPs/ROPs.

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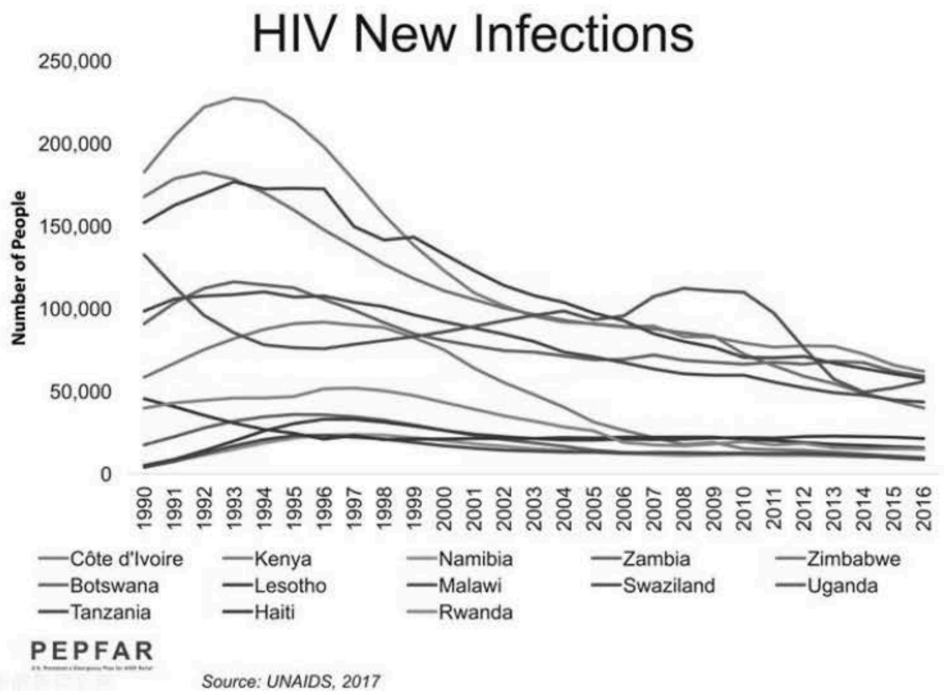
Figure 7: Number of New HIV Infections in Select PEPFAR-Supported Countries, 1990–2016



Overall, there has been a significant decrease in the rate (incidence) of new HIV infections during the last 15 years, although the decline in new infections varies significantly by country (Figure 7).

In sub-Saharan Africa, where the epidemic is the most costly and deadly, results vary from country to country due to the history of the epidemic and coverage of specific interventions. Effective interventions were not advanced at the same rate and in the same manner, so changes in new infections and AIDS-related mortality differ across countries (Appendix W).

Decreasing the absolute number of new infections – and not only incidence – is essential for both epidemic control and fiscal sustainability, as it drives down the burden of disease and the cost of caring for HIV-positive individuals. While the incidence rate has declined in most PEPFAR countries, the size of the populations most at risk for HIV infection, especially young women, has substantially expanded in the last 20 years. Overall populations have grown, especially those under 25 years of age, nearly doubling since the beginning of the HIV pandemic. Therefore, even with incidence declining by 50 percent, only a steady state – not a decline – would be achieved with the doubling of the population at risk.

This is particularly the case in sub-Saharan Africa where, due to high fertility rates and improved child survival, the population of 15- to 24-year-olds will have doubled by 2020 from the beginning of the epidemic on the continent (Figure 9). This is further illustrated by a single country, Zambia (Figure 10), and this is replicated in country after country. With the significant increases in the total population of sub-Saharan Africa, specifically the increase in young people, we have reached a critical juncture. In this context, our programs must continually be even more effective just to maintain the status quo and must significantly increase their impact to control the pandemic. This is why PEPFAR

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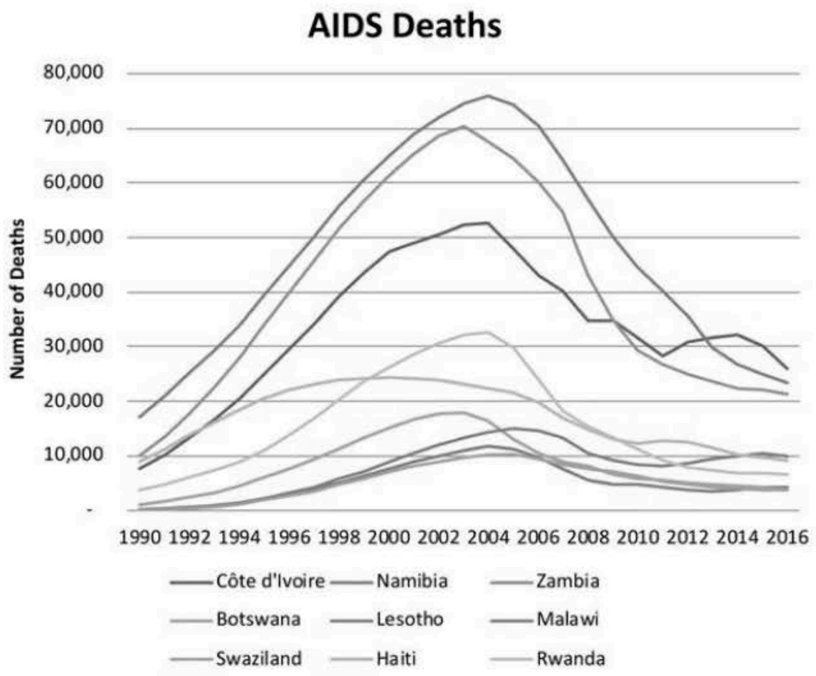
Figure 8: Number of AIDS-Related Deaths in Select PEPFAR-Supported Countries, 1990–2016



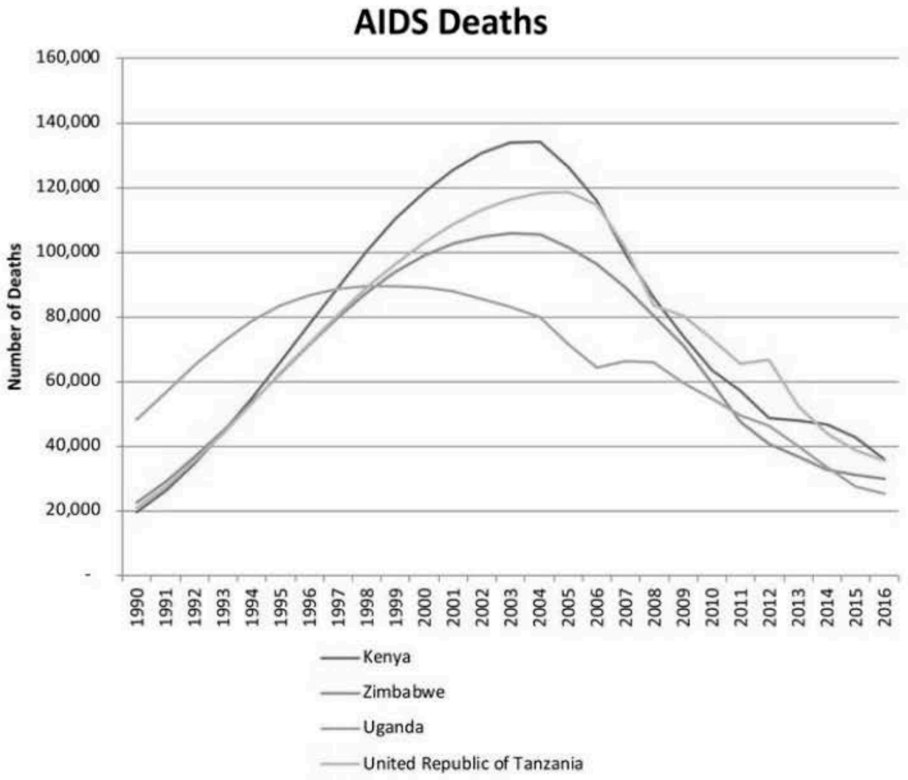


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Figure 9: Youth Bulge in Sub-Saharan Africa

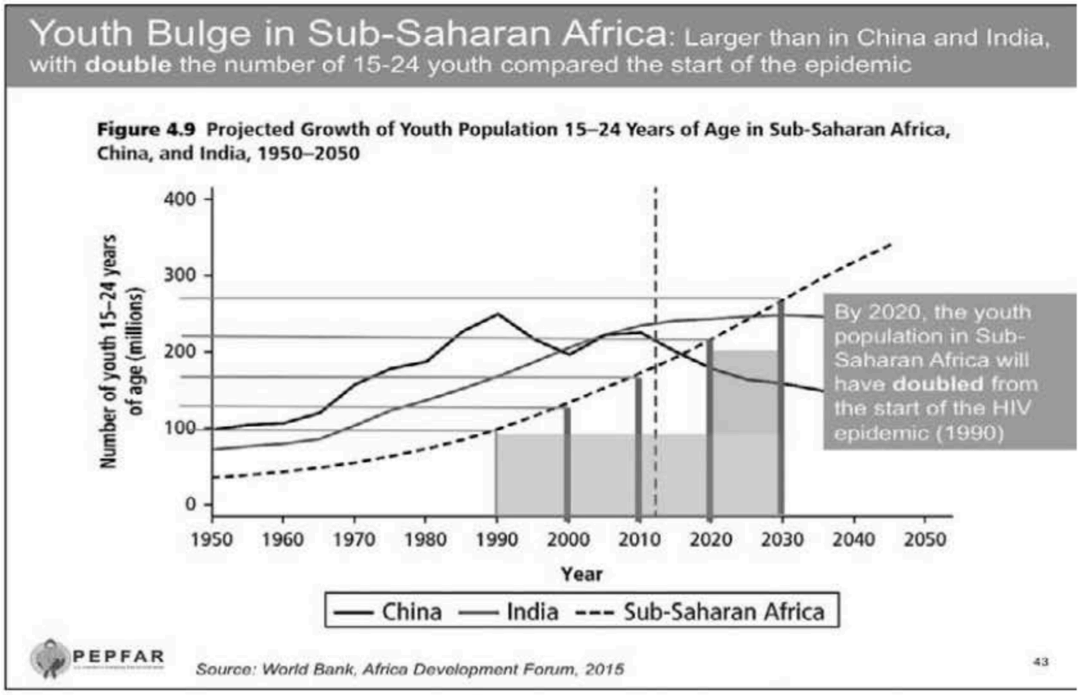
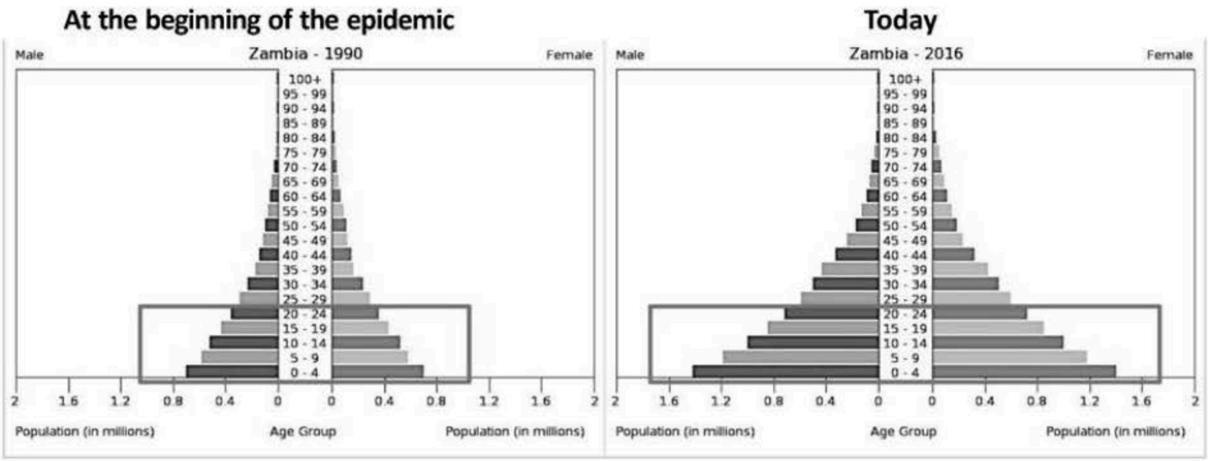


Figure 10: Youth Bulge in Zambia



Young Men Population: 781,000
Young Men PLHIV: 38,000
Young Women Population: 772,000
Young Women PLHIV: 66,000

Young Men Population: 1.6 million
Young Men PLHIV: 48,000
Young Women Population: 1.6 million
Young Women PLHIV: 77,000

PEPFAR Source: US Bureau of the Census, 2017 & UNAIDS, 2017

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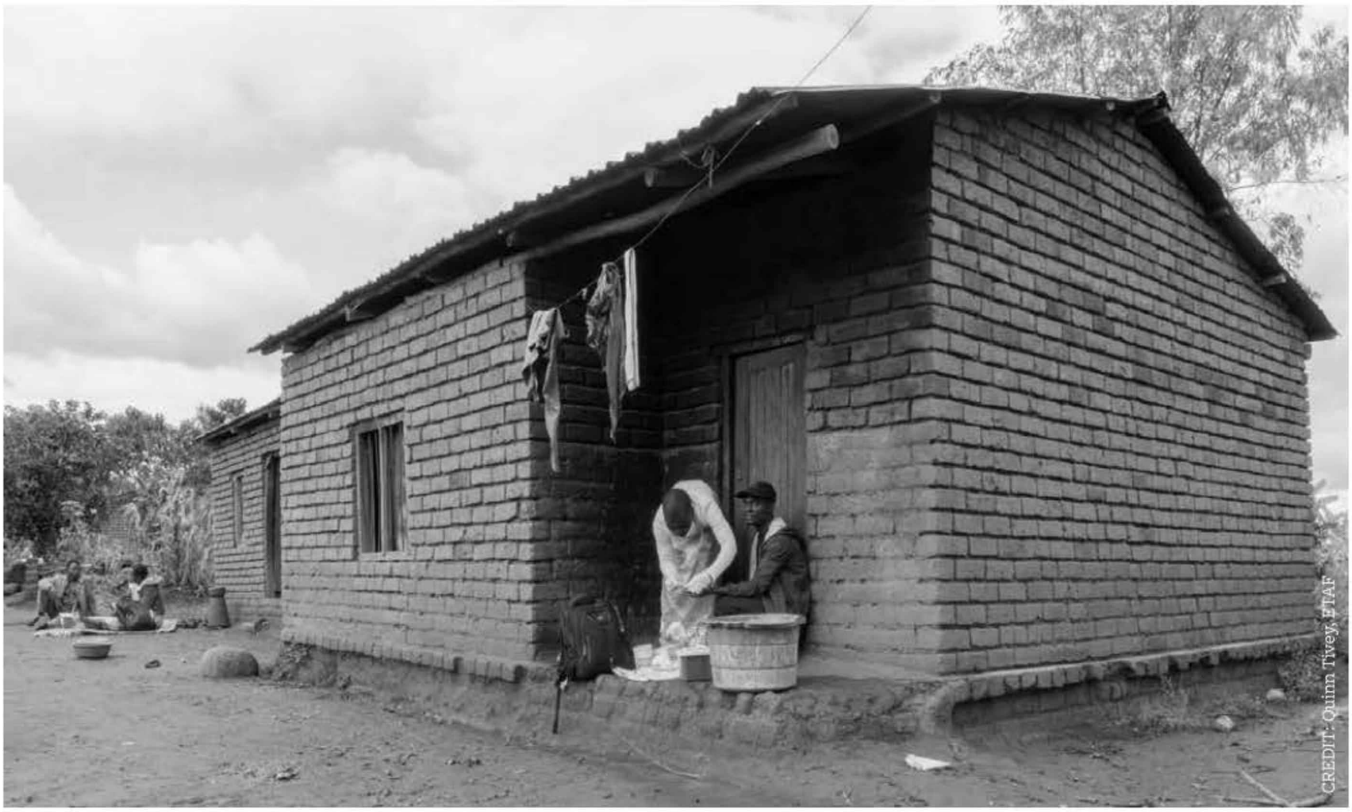

A community health worker tests a man for HIV at his home.

continues to expand prevention for young women and is focused on diagnosing and treating young men to break the transmission cycle.

PEPFAR will continue increasing program effectiveness through the enhanced use of facility-level data disaggregated by sex and five-year age bands. We will continue refining our focus on the geographic areas and populations most in need of services to prevent new HIV infections in sub-Saharan Africa, which are otherwise projected to increase by an additional 25–26 million by 2030.[3] Such an increase would nearly double the current cost globally to provide lifesaving services to all of those in need. The escalating total cost of treatment to save lives associated with such an increase cannot be sustained by any combination of financing from the host country, The Global Fund to Fight AIDS, Tuberculosis and Malaria (Global Fund), PEPFAR, or increased partner country contributions. The absolute key to sustainability is to decrease the number of new infections.

We are at a moment in time when we have all of the tools necessary to change the course of the epidemic, and we are beginning to see these promising results in our PHIA surveys (Figures 11 and 13). However, we must continually use granular data and laser-focus every dollar spent to ensure we are addressing those in greatest need, especially the younger populations, as shown in Figure 12. Otherwise, we will face an epidemic that will once again spiral out of control, reversing the progress achieved through our investments to date. It is a quarter-by-quarter, community-by-community intense focus on prevention and treatment that will result in persistent control of the pandemic.

[3] UNAIDS. (2014). Fast-Track – Ending the AIDS Epidemic by 2030. Geneva.

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Figure 11: Progress to 90-90-90 in Adults

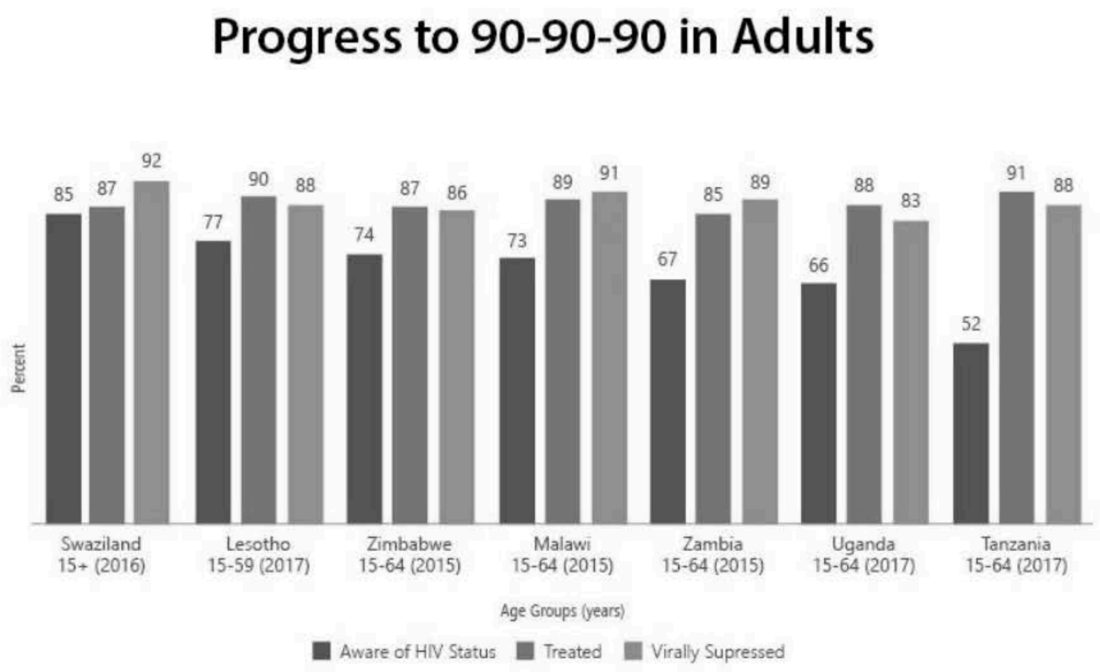

Figure 12: Progress to 90-90-90 in Adolescents and Young Adults (15–24-year-olds)

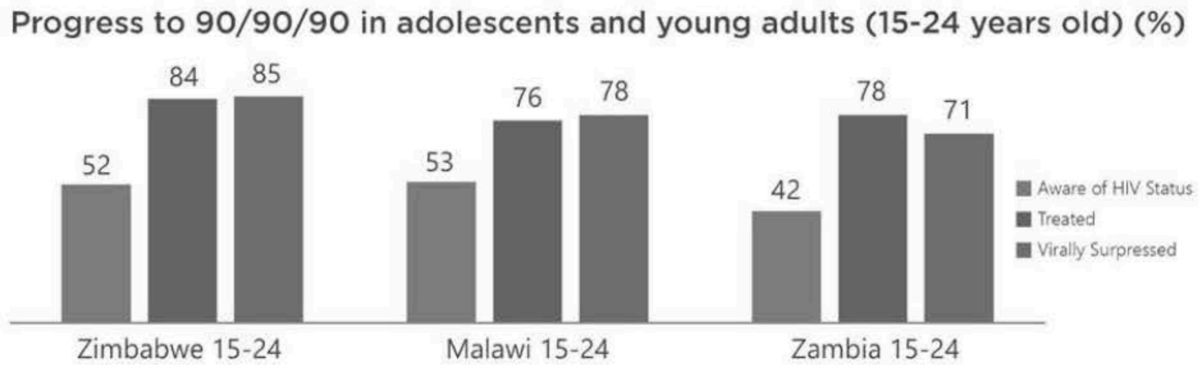

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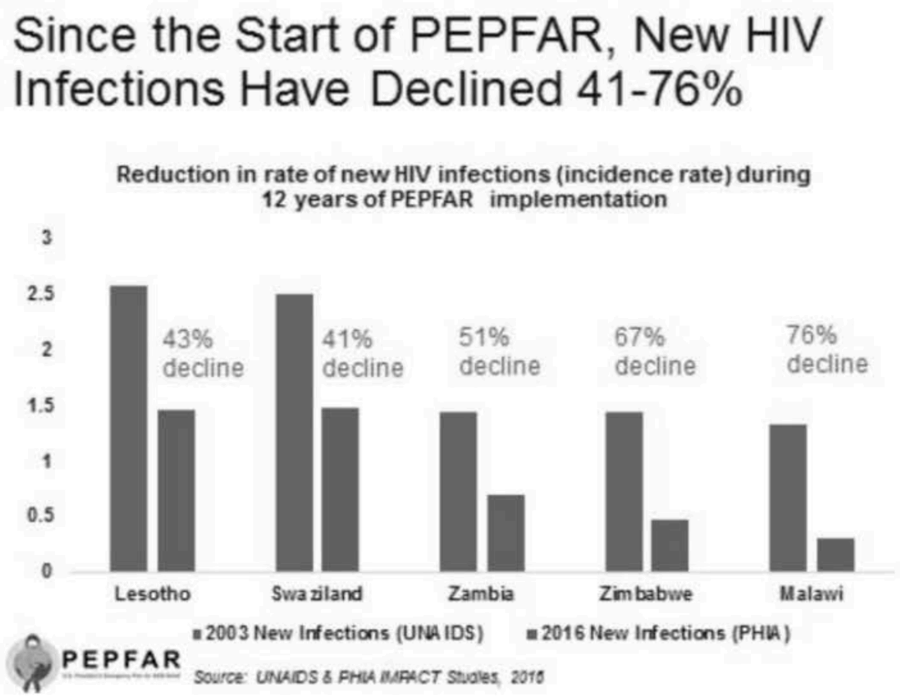
Figure 13: Decline in New HIV Infections in Select PEPFAR-Supported Countries



HIV Infections Averted Due to PEPFAR and Global HIV Response

Modeled data suggest that a cumulative total of nearly 16 million HIV infections globally have been averted since the beginning of the epidemic, including 11.3 million HIV infections in sub-Saharan Africa, due to PEPFAR and the global HIV/AIDS response.

Validating the Models

Due to PEPFAR investments in the PHIAs, the HIV incidence rate is now measured directly and estimated more precisely. Currently, six of the 13 high-burden countries have new incidence measures as well as clear prevalence data disaggregated by both age and sex. Among the remaining seven high-burden countries, the PHIAs are planned or ongoing.

PEPFAR funded a comprehensive survey in 2011 that provides a clear baseline in Swaziland and a comparator of current results and progress of PEPFAR investments over a five-year period (Figure 14). Figure 14 compares findings from the two PEPFAR-supported SHIMS conducted in 2011 and 2016–17, respectively. It shows a dramatic decline in new infections in women under age 25 as seen by the decreases in early prevalence and a real improvement in life expectancy of HIV-positive adults who are mothers and fathers, teachers, and nurses thriving in their communities. It also captures the impact of PEPFAR over the past five years, which we are focused on accelerating.

PEPFAR continues to model partner countries' results with the most recent national data available from UNAIDS using the Goals model, which is a method for costing and resource allocation that can be used in the development of national HIV/AIDS strategic plans and investment frameworks.[4]

[4] Stover, J., Walker, N., Garnett, G., Salomon, J., Stanecki, K., Ghys, P., ... Schwartlander, B. (2002). Can we reverse the HIV/AIDS pandemic with an expanded response? The Lancet, 360, 73–77.

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PEPFAR beneficiaries in Mozambique.

Figure 14: Dramatic Declines in HIV Prevalence and Success of ART in Swaziland

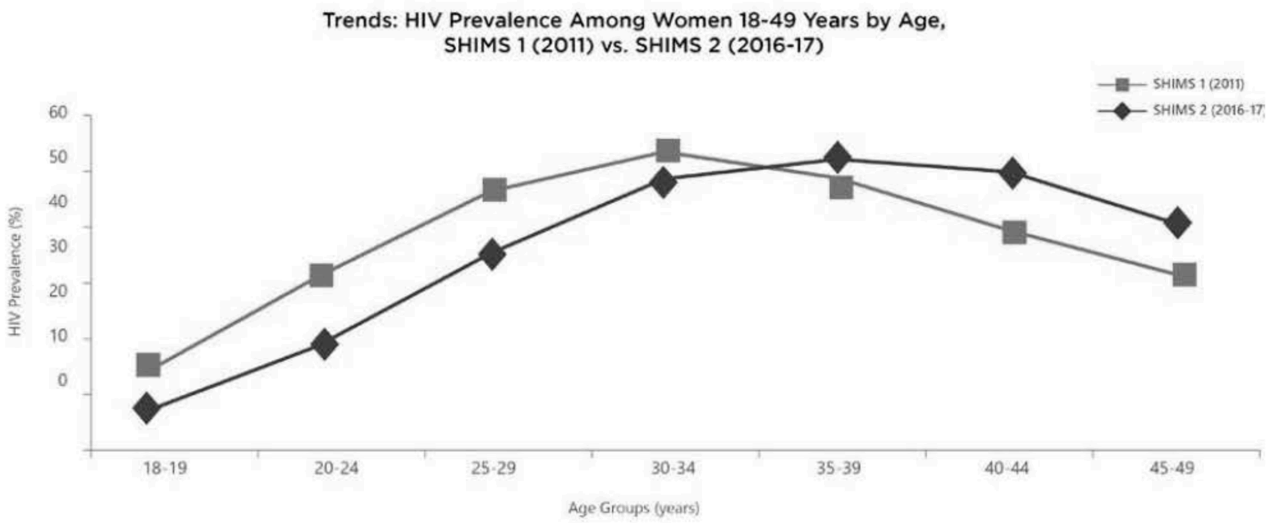

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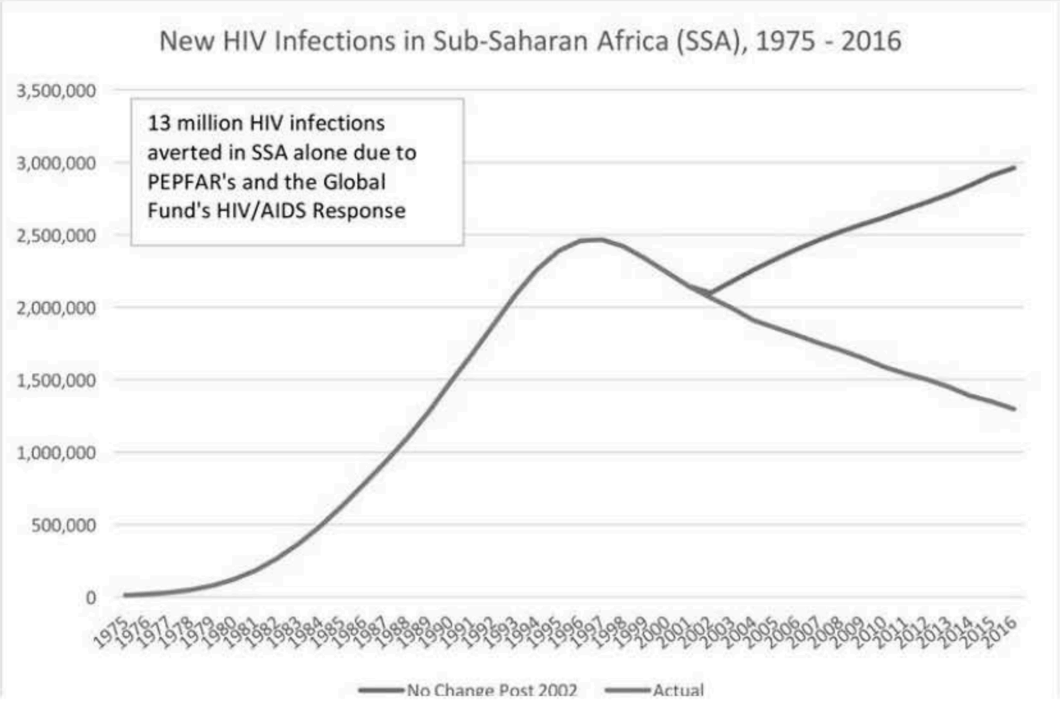
Figure 15: New HIV Infections in Sub-Saharan Africa, 1975–2016



Global Prevalence: Refining PEPFAR's Impact and Progress Toward Epidemic Control and Implications of Future Costs for a Sustained HIV/AIDS Response

According to UNAIDS, eastern and southern Africa accounted for 44 percent of the 1.8 million new HIV infections globally in 2016, which is a decline of 54 percent since 2006 (Figure 15). Reducing the absolute number of annual new HIV infections is essential, particularly in eastern and southern Africa where these must fall below 500,000. Beyond saving untold numbers of lives, this will reduce the future costs required to sustain the HIV/AIDS response.

The report notes that focusing on populations that are underserved and at a higher risk of HIV infection is critical to ending the AIDS epidemic. This principle underpins the PEPFAR 3.0 Strategy, which aims to do the right things, in the right places, in the right way, and at the right time to achieve maximum impact.

PEPFAR invests in more than 50 countries around the globe through both country and regional platforms. Among these, 13 high-burden countries have the greatest potential to reach epidemic control by 2020. It is in these 13 countries where PEPFAR is intensifying our focus to create the road map to reach epidemic control and demonstrate the requirements needed to maintain it. PEPFAR will continue to support the other countries utilizing available resources to focus our investments. Figure 17 shows the progress needed to get new infections below the total number of deaths among all HIV-positive individuals.

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To accelerate progress toward achieving epidemic control, PEPFAR supports programs that significantly decrease transmission by HIV-positive individuals by providing them with lifesaving ART, advancing HIV prevention through risk avoidance and risk prevention strategies for those who are HIV-negative, and expanding voluntary medical male circumcision (VMMC) for HIV-negative young men. Pursued in combination, these strategies will reduce the transmission of new infections, and through using the age and sex disaggregated data will ensure all groups decrease their transmission. Through these efforts, 13 countries can reach greater than 70 percent ART coverage by the end of fiscal year (FY) 2018.

Aligning with the PEPFAR 3.0 Strategy, we continue to show outcomes and impact through our comprehensive PHIAs, which measure HIV prevalence, incidence, historic mortality, and service coverage down to the household level. Figure 18 shows the timeline and countries where these surveys are being conducted and where we are directly measuring impact.

According to the most recent PHIA results, four PEPFAR countries have greater than 70 percent of HIV-positive people with known status, and greater than 85 percent of those currently active on treatment. However, coverage is not equal across genders and age groups. Coverage is particularly lagging among children who are currently well, asymptomatic young women and young men who are thereby not accessing health facilities for diagnosis and treatment.

Strengthening Program Cost Effectiveness

Efficiency

For the first time in modern history, we have the tools to achieve epidemic control. As PEPFAR invests in new innovations, we will

Figure 16: Swaziland – Pathway to Reaching Epidemic Control

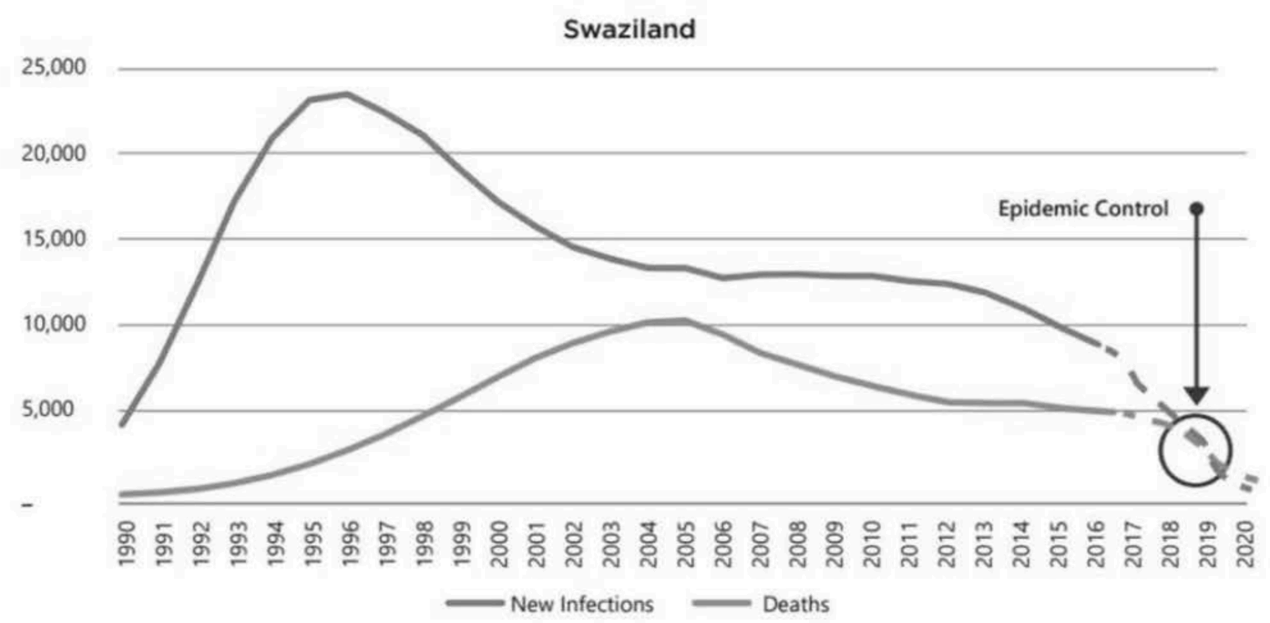

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Figure 17: Comparing Annual New HIV Infections and Annual Total Deaths Among HIV-Positive Individuals in 13 High-Burden Countries

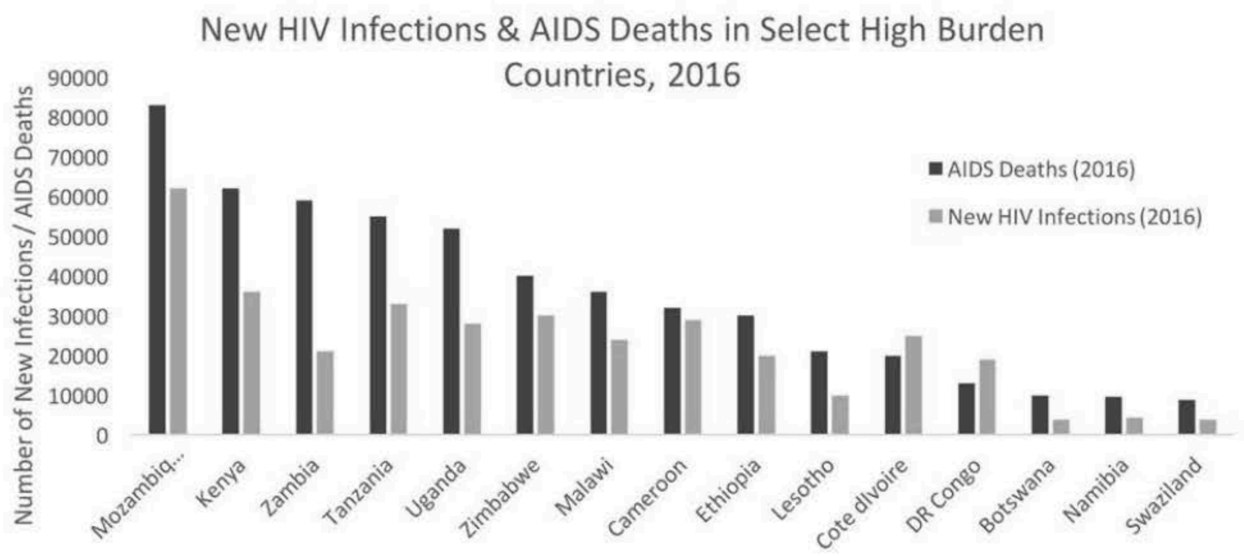
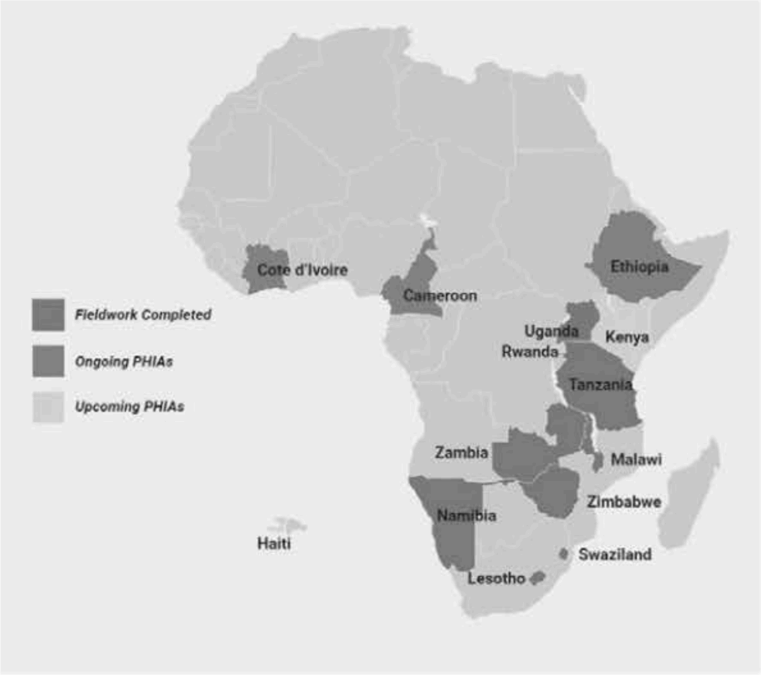


Figure 18: Implementation Timeline and Status of HIV Impact Assessments



Completion Date (or expected)	
Malawi	2016
Zambia	2016
Zimbabwe	2016
Lesotho	2017
Swaziland	2017
Tanzania	2017
Uganda	2017
Cameroon	2018
Cote d'Ivoire	2018
Ethiopia	2018
Namibia	2018
Haiti	TBD*
Kenya	TBD*
Nigeria*	TBD*

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CREDIT: USAID

A group of young mothers receive health training in India.

continue scaling our programs using these tools toward reaching this goal in all PEPFAR countries. This attainable progress requires that countries demonstrate political will and execute their own responsibilities, including by ensuring that their existing funding from other bilateral and multilateral organizations is well coordinated with funding from PEPFAR.

The Right Policies Are Fiscally Responsible

The challenge for the world is to increase the number of people on treatment and reach the UNAIDS 90-90-90 treatment targets while at the same time working within a constrained budget environment. PEPFAR continues to generate significant savings to achieve greater goals. The program adopted a number of policies and innovations that enable existing resources to go further. These policies include Test and Start, multi-month ART prescriptions, same-day initiation, and differentiated service delivery.

In 2017, PEPFAR worked to ensure full and complete rollout of Test and Start policies. While it may seem counterintuitive to initiate lifetime treatment as a cost control measure, quicker initiation of treatment pays off in the long run. There are many benefits to starting treatment as soon as an individual tests positive.

First, and most importantly, patients who are on effective HIV treatment are not sick, do not require hospitalization, remain employed, and are more productive, which also has positive economic benefits. Critically, there is also the prevention benefit of a person who is virally suppressed. Between 60 and 80 percent of incidence reductions necessary for epidemic control will come from prevention benefits resulting from treatment.

There are also other health systems savings resulting from early treatment, including lower hospitalization costs and lower levels of TB and other opportunistic infections. Finally, there are additional societal savings. Households with members on treatment have higher levels of education and incomes, and there are fewer OVC.

Test and Start also enables countries to adopt same-day initiation of ART. While same-day initiation is not appropriate in every case, wider use of the policy streamlines ART costs and prevents loss to follow-up costs. The cost of finding patients who test positive and are lost to follow-up is more expensive than the cost of putting them on treatment in the first place.

Finding patients who are more recently infected and healthier also means that initiation costs are lower and less complex, and drug regimens more tolerated. Same-day initiation also helps the patient become virally suppressed sooner, and generates more prevention benefits and other health systems savings.

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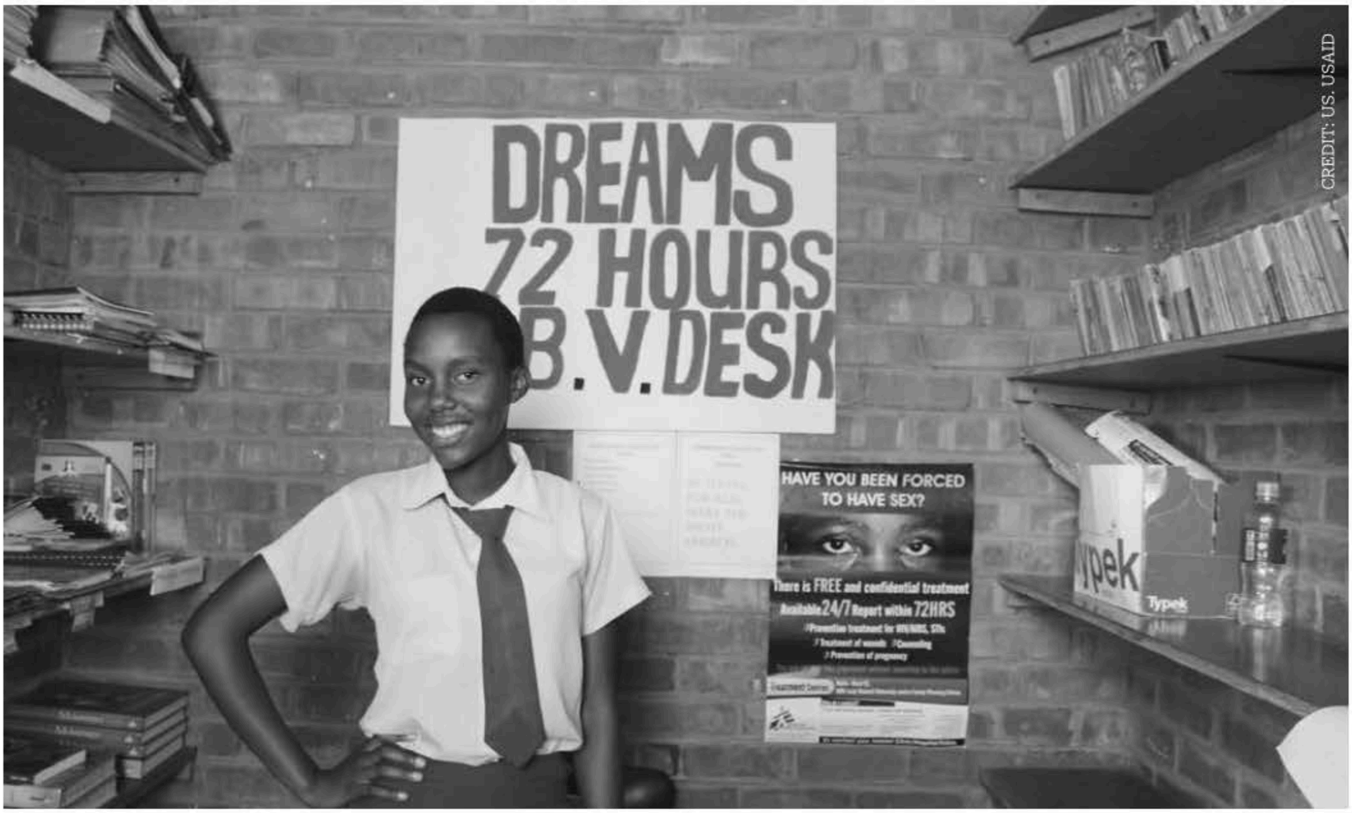
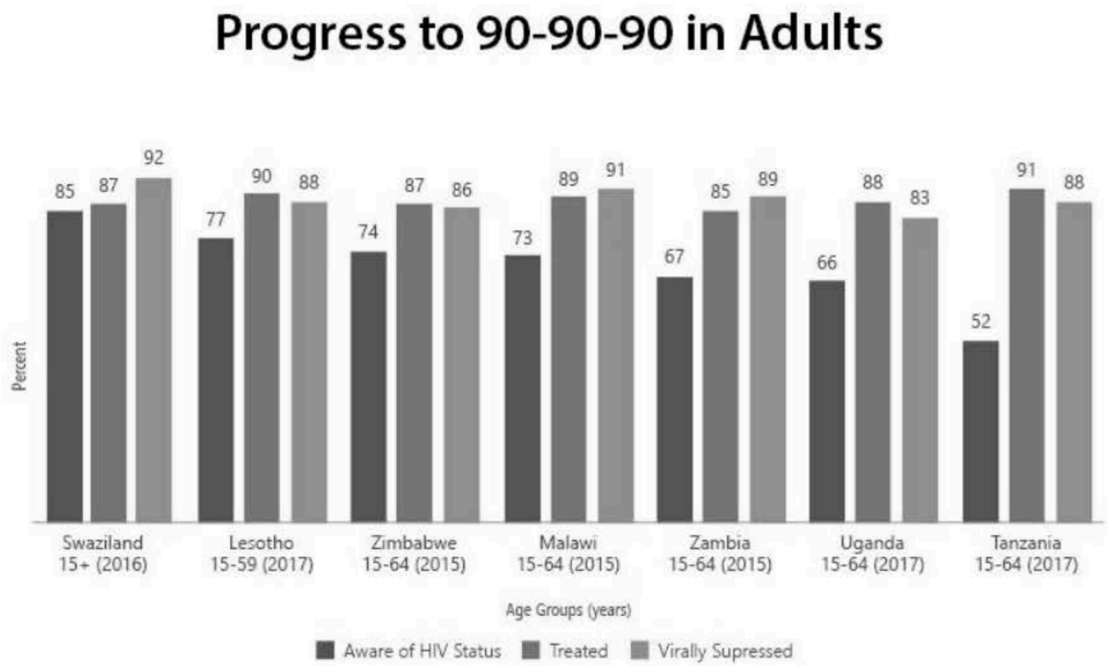


CREDIT: US. USAID

A young girl learns about preventing gender-based violence in Zimbabwe.

Figure 19: Accelerating Epidemic Control in Swaziland, Lesotho, Zimbabwe, Malawi, Zambia, Uganda, and Tanzania



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In FY 2017, PEPFAR launched a coordinated push for implementation of differentiated models of care. As more people become stable on treatment, their infection can be managed as a chronic disease, and they can be transitioned to a treatment regimen that has fewer clinic and pharmacy visits to collect their antiretroviral medications (ARVs). Existing clinic staff can, in turn, maintain larger caseloads of clients, enabling these medical facilities to implement Test and Start without significantly increasing costs.

U.S. ambassadors posted around the globe have been critical in promoting and ensuring the adoption and implementation of the key policies by the host countries in which PEPFAR works, allowing our dollars to be more effective. The right policies not only save lives, but also make our program more impactful, dollar for dollar.

New Drug Regimens and Other Commodity Savings

PEPFAR is also supporting a rapid rollout of new and more effective regimens based on Dolutegravir, a new integrase inhibitor. Dolutegravir (DTG) is cheaper, more tolerable, and leads to better results including faster viral suppression. There is a virtuous circle created by DTG's low side effect profile, which makes adherence easier. Easier adherence and fewer side effects mean a more rapid adoption of differentiated care and more models of community care. It will be critical to utilize these more effective regimens that are more easily tolerated, especially as we start clients on treatment who are early in the progression of the disease and feeling well. Regimens must be well-tolerated to ensure people stay on their effective treatment.

CREDIT: Quinn Tivey, ETAF

Five women and one man wait to be seen at a mobile health clinic in Malawi. The photo captures the ratio of women to men being tested for HIV at our mobile health clinics prior to the implementation of a special male-focused program. In 2017, the ratio had been reduced to 1:1.5.

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Due to faster viral suppression, ART prevention benefits are also felt quicker with the use of DTG. The drug's wide applicability – including for patients currently on second-line regimens – simplifies supply chain regimes. It creates very low resistance, which should allay concerns that too many people on treatment could lead to the rise of drug-resistant strains of HIV.

PEPFAR is also working to lower the costs of other purchases, most notably the cost of laboratory reagents. For example, PEPFAR has achieved impressive reductions in the cost of viral load tests, in some cases from $40 per test to as low as $15. Further future reductions are possible. In addition, with fewer clinic visits, fewer laboratory tests are needed as PEPFAR works hard to eliminate unnecessary tests. In fact, with Test and Start, CD4 counts are no longer necessary to determine the initiation of ART. PEPFAR is scaling back support to CD4 testing, which is generally needed in fewer cases, freeing up resources for the expansion of viral load monitoring to ensure clients remain virally suppressed.

Toward Better Cost Data

In 2017, PEPFAR launched a new effort to improve internal budget practices, collecting and analyzing more accurate cost data. The existing Expenditure Analysis system was updated to reflect the fixed and variable costs of treatment and support. In partnership with others, including the Global Fund and the Bill & Melinda Gates Foundation (Gates Foundation), PEPFAR launched new efforts to better align resources, avoid duplication, and improve the cost analysis of HIV treatment and prevention programming.

The Gates Foundation has taken the lead on developing the Global Health Cost Consortium (GHCC). GHCC is providing more comprehensive and analytic data for planning COP/ROP budgets and discussions with partner country ministries of health and finance on shared responsibilities of the HIV response. This collaboration will be a multiyear effort to better align resources, change financial systems, and drive down program costs. Through an accounting of investments made "above the site" level described in Section 4, PEPFAR is also gathering better information on the costs and outcomes of our systems investments, which enable a more purposeful transfer of responsibility from PEPFAR and international donors to partner countries. Annually PEPFAR invests more than $700 million above-site in health systems required for effective HIV/AIDS services but also needed for an effective global health security agenda.

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Table 1: Reported PEPFAR Expenditures by Program Area for Select Core Programs, 2016 Fiscal Year (October 2015 to September 2016), Millions

Country	Facility-based Care Treatment, and Support	Comunity-based Care Treatment, and Support	PMTCT	VMMC	HTC	Laboratory	OVC	Key Population Prevention – person whoinjects drugs	Key Population Prevention	Strategic Information	Total Core Programs
Angola	$4.73	$1.67	$0.63		$1.94	$2.96		$0.11	$0.58	$0.53	$12.88
Botswana	$12.10	$5.70	$1.48	$5.87	$8.34	$4.48	$2.45		$0.79	$1.90	$43.10
Burma	$2.16	$0.14	$0.06		$0.95	$1.22	$0.04	$0.11	$1.56	$0.35	$6.60
Burundi	$1.93	$0.22	$3.95	$0.02	$1.78	$0.24	$0.08	$0.05	$0.27	$0.34	$8.89
Cambodia	$1.65	$0.60	$0.34		$0.88	$0.62		$0.29	$3.04	$0.08	$7.51
Cameroon	$10.41	$1.26	$9.52		$2.21	$1.79	$0.97		$1.29	$0.39	$27.85
Costa Rica	$0.17	$0.05			$0.07	$0.01			$0.20	$0.04	$0.53
Cote d Ivolre	$35.81	$8.42	$11.11		$8.42	$17.46	$8.48		$2.19	**$1.39**	$93.29
Democratic Republic of Congo	$2.40	$0.93	$.023		$1.17	$0.96		$0.18	$1.70	$0.72	$8.28
Dominican Republic									$0.00	$0.02	$6.95
Ethiopia	$63.57	$4.89	$13.28	$0.85	$9.03	$19.38	$11.17		$5.41	$11.11	$138.69
Ghana	$1.08	$0.04	$0.01		$0.93	$1.40			$3.47	$0.02	$6.95
Guyana	$1.34	$0.42	$0.03		$0.29	$0.23	$0.38		$0.58	$0.03	$3.30
Haiti	$28.52	$7.61	$7.25		$11.52	$15.15	$6.12	$0.34	$1.99	$1.44	$79.94
India	$1.81	$0.43	$0.60		$0.46	$1.14	$1.27	$1.47	$2.91	$0.84	$10.93
Indoesia	$1.45	$.083			$1.08	$0.87		$0.55	$2.46	$0.46	$7.71
Kenya	$211.48	$13.65	$24.25	$15.25	$43.76	$25.57	$40.04	$2.24	$7.51	$10.68	$394.43

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Table 1: Reported PEPFAR Expenditures by Program Area for Select Core Programs, 2016 Fiscal Year (October 2015 to September 2016), Millions

Country	Facility-based Care Treatment, and Support	Comunity-based Care Treatment, and Support	PMTCT	VMMC	HTC	Laboratory	OVC	Key Population Prevention – person whoinjects drugs	Key Population Prevention	Strategic Information	Total Core Programs
Lestho	$11.30	$1.45	$3.38	$5.08	$5.29	$3.19	$3.00		$0.22	$0.49	$33.41
Malawl	$33.22	$4.63	$6.91	$9.41	$13.23	$9.57	$3.24	$0.04	$0.47	$1.79	$83.89
Mozambique	$92.41	$14.87	$25.32	$19.00	$16.09	$29.85	$12.97	$0.18	$1.99	$4.28	$216.95
Nambia	$9.76	$3.37	$1.33	$6.11	$5.34	$4.81	$3.55	$0.04	$0.47	$1.79	$36.93
Nigeria	$148.74	$22.80	$34.86		$58.15	$36.18	$37.74	$4.33	$19.48	$4.65	$366.92
Papua Ne Gu°inea	$0.83	$0.02			$0.44	$0.19			$1.65	$0.08	$3.24
Rwanda	$31.63	$2.56	$7.84	$1.71	$5.33	$10.64	$8.14	$0.01	$2.17	$0.24	$70.29
South Africa	$129.02	$24.17	$20.20	$52.24	$67.31	$8.38	$44.66	$0.70	$5.94	$10.49	$363.18
South Sudan	$2.84	$0.16	$1.66		$3.03	$0.59	$0.56	$0.08	$2.10	$0.06	$11.08
Swaziland	$11.62	$2.31	$2.81	$3.58	$3.88	$2.50	$7.74		$0.48	$1.67	$36.61
Tanzania	$122.67	$30.77	$41.09	$23.42	$37.25	$15.91	$32.25	$1.17	$5.54	$5.65	$315.72
Uganda	$123.78	$15.06	$25.82	$23.25	$16.25	$21.62	$23.35	$0.12	$5.59	$9.86	$264.69
Ukraine	$4.56	$1.49	$0.02		$1.33	$0.66	$0.00	$1.67	$0.32	$6.74	$16.78
Vietnam	$23.03	$0.23	$0.47		$4.03	$4.01		$2.42	$2.04	$1.29	$337.53
Zambia	$112.45	$13.43	$20.84	$13.33	$16.38	$27.95	$18.28	$.012	$2.39	$2.82	$227.99
Zimbabwe	$41.29	$2.56	$4.34	$18.48	$9.69	$6.83	$8.63		$0.62	$2.94	$95.39

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FOCUSING PREVENTION FOR IMPACT

As of September 30, 2017, PEPFAR had supported more than 15.2 million men and boys in eastern and southern Africa with substantial protection from HIV infection through the provision of VMMC. In FY 2017 alone, PEPFAR supported the largest single-year increase (nearly 3.5 million) in VMMC results since the program's inception.

This prevention intervention is durable and has at least 60 percent efficacy, making it a highly effective and efficient prevention intervention for men and boys. As nearly 50 percent of the VMMC performed are for school-age boys under 14 years, it will take 10 years to realize the full prevention benefits from this intervention when sexual risk of HIV begins to increase for men. We continue to focus the intervention on 15–29-year-old men, which remains a difficult age group to reach, both for VMMC and for HIV testing for diagnosis and treatment.

To address the alarming rates of gender-based violence (GBV) faced by children and the disproportional risk of HIV infection among adolescent girls and young women (AGYW) in many African countries, PEPFAR launched the Determined, Resilient, Empowered, AIDS-free, Mentored and Safe (DREAMS) public-private partnership (PPP) on World AIDS Day 2014. Every year, 360,000 AGYW are infected with HIV – nearly 1,000 a day. GBV is a significant reason why, affecting millions of AGYW. Girls who experience violence are up to three times more likely to be infected with HIV or other sexually transmitted infections. On World AIDS Day 2017, we reported that in the 10 African countries implementing DREAMS programs, nearly two-thirds (65 percent) of the highest HIV-burden communities or districts achieved a 25–40 percent or greater decline in new HIV diagnoses among young women since 2015. Reaching those at most risk, including adolescent girls and young women, men, and key populations, is critical to ending the HIV/AIDS epidemic.

In order to reach epidemic control, we must address the underlying social and cultural issues, especially unequal human rights and stigma and discrimination, that prevent people from accessing HIV prevention and treatment services. When any person is stigmatized or unable to access services due to discrimination, the health of everyone in the community is threatened and the epidemic continues to expand rather than contract. The following section focuses on PEPFAR's commitment to prevention for impact by ensuring that those at most risk of acquiring HIV are able to protect themselves from infection.

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Voluntary Medical Male Circumcision is a focus area for USAID's AIDSFree Project.

CREDIT: Lisa Russell, Governess Films and AIDSFree Project

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PEPFAR and Prevention Interventions

Prevention, treatment, and care have been the three pillars of PEPFAR programming since our inception in 2003. This comprehensive approach was mandated by Congress in PEPFAR's initial authorizing legislation and was included in each subsequent reauthorization. Prevention services are grouped together in a comprehensive package for maximum impact. This package can include our continued focus on the prevention of mother-to-child transmission (PMTCT) of HIV followed by condom programming, behavioral and structural interventions, risk avoidance and reduction, pre-exposure prophylaxis (PrEP) with ARVs, HTS, and VMMC for HIV-negative young men. These prevention activities target those most at risk of HIV acquisition, focused on risk groups and areas of high HIV burden.

Global results show dramatic improvement in preventing babies from being born with HIV, but much less of an impact on reducing new adult infections, indicating a need to refocus on prevention in young adults. PEPFAR was enormously successful in PMTCT implementation, dramatically decreasing new pediatric infections and helping mothers with HIV live healthy, productive lives. These programs will continue to be a cornerstone of PEPFAR. Protecting and ensuring that babies remain HIV-free resulted in significant improvements in under age 5 survival rates, reflected in the impressive progress achieved toward the Millennium Development Goals. Also, a continued critical focus of PEPFAR has been ensuring that HIV-positive mothers remain on lifesaving treatment, for their own health and that of their babies. The next challenge is keeping these babies HIV-free as they age into adolescents and young adults.

PEPFAR aims to meet that challenge and is co-leading with UNAIDS *Start Free Stay Free AIDS Free*, a super-fast-track framework for ending AIDS among children, adolescents, and young women by 2020. This means that PEPFAR is working to ensure that infants are born HIV-free, AGYW stay HIV-free, and children and adolescents living with HIV have access to the lifesaving treatment and care needed for them to be AIDS-free. This collaboration with key partners is building on the progress of the *Global Plan Towards the Elimination of New HIV Infections Among Children by 2015 and Keeping Their Mothers Alive* as well as accelerating key initiatives (Figure 20).

The collective global framework aims to achieve these goals: (1) Start Free – provide 95 percent of pregnant women living with HIV with lifelong HIV treatment by 2018 and reduce the number of children newly infected to less than 40,000 by 2018 and 20,000 by 2020; (2) Stay Free – reduce the number of new HIV infections among adolescents and young women to less than 100,000 by 2020 and provide VMMC to 25 million additional men, especially young men

In Zimbabwe, a group of men in the community break into a traditional dance called "hoko" as they celebrate the safe return of new graduates who had just been circumcised, and their successful completion of the rite of passage.

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Figure 20: The Goals of the *Start Free Stay Free AIDS Free* Framework[5]

ages 10–29, by 2020; and (3) AIDS Free – provide ART to 1.6 million children (0–14 years) and 1.2 million adolescents (15–19) living with HIV by 2018 and to 1.4 million children (0–14) and 1 million adolescents (15–19) with HIV by 2020.

Since PEPFAR's inception in 2003, a total of nearly 2.2 million infant HIV infections have been averted, allowing these infants to Start Free, thanks to PEPFAR support. Nearly half of that progress has been achieved since 2013. In 2017, PEPFAR supported HTS for 11.2 million pregnant women; 94 percent of the women who tested positive for HIV were then provided with ART.

Through our DREAMS PPP with the Gates Foundation, Girl Effect, Johnson & Johnson, Gilead Sciences, and ViiV Healthcare, PEPFAR is helping girls stay free of HIV infection and grow into a Determined, Resilient, Empowered, AIDS-free, Mentored, and Safe woman.

PEPFAR expanded DREAMS programming in the 10 original countries in 2017.

As a result, 41 of the highest HIV-burden districts (65 percent) have achieved a decline in new diagnoses among AGYW ages 15–24 by more than 25 percent since 2015; 14 of those districts have had a decline of greater than 40 percent. Five more countries are implementing DREAMS in 2018, reaching even more AGYW with interventions that go beyond the health sector to address the structural drivers that increase girls' HIV risk, including poverty, gender inequality, sexual violence, and lack of access to an education. Among 10–14-year-old girls, the program has focused on risk avoidance and strengthening families and communities to embrace and protect their girls.

In addition, of more than 3.4 million VMMCs that PEPFAR supported for HIV prevention

[5] UNAIDS. (2016). Start Free, Stay Free, AIDS Free: A Super-Fast-Track Framework for Ending AIDS Among Children, Adolescents and Young Women by 2020.

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in eastern and southern Africa in 2017, nearly 1.6 million were among men ages 15-29. Male circumcision has the potential to prevent millions of new infections, preserve millions of lives, and save billions of dollars in averted HIV treatment costs. Importantly, this procedure brings men – some for the first time since childhood – into health services and is a key intervention for reducing risk of HIV transmission to AGYW.

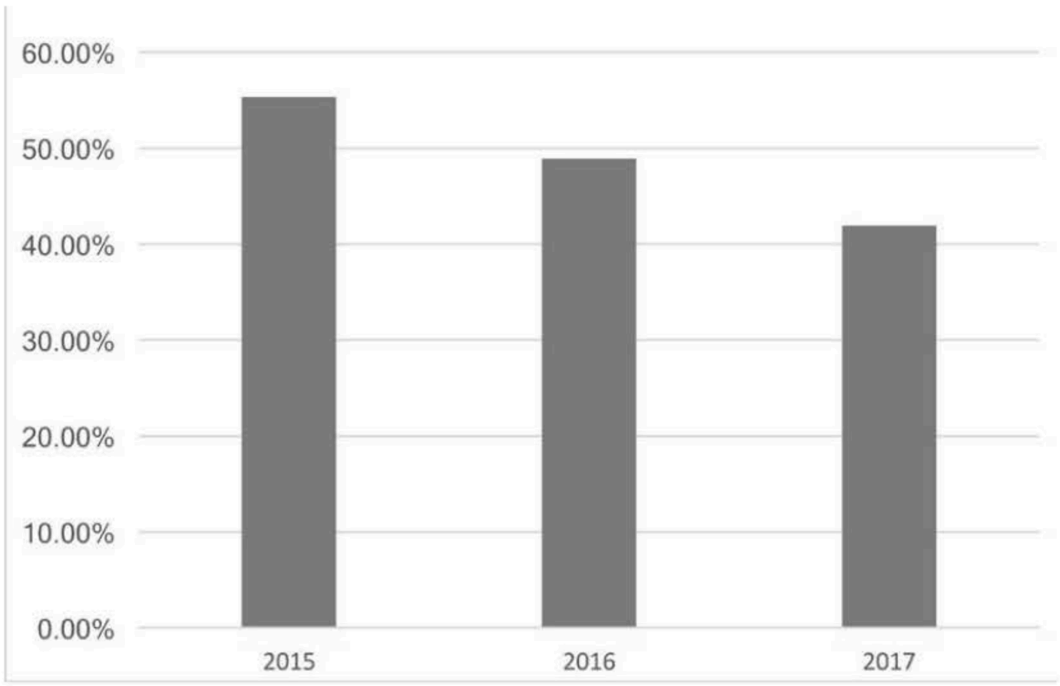
Continuing Prevention of Mother-to-Child Transmission (PMTCT)

PEPFAR remains fully committed to working toward the elimination of new HIV infections among children and keeping their mothers alive. A cumulative total of 2.2 million infant HIV infections have been averted since the beginning of PEPFAR – with nearly half of that progress achieved since 2013. That means babies are being born HIV-free, and their mothers are staying healthy and AIDS-free to protect and nurture them.

Over the past two years, the rate of HIV testing of pregnant women presenting to PEPFAR-supported sites has increased from 93.3 percent to 99.8 percent. In addition, the proportion of pregnant women newly diagnosed with HIV among all pregnant women who tested positive has decreased from 53 percent to 43 percent. This shows that more women are entering pregnancy with known status and already on antiretroviral therapy to protect their health and that of their infants, and suggests a decrease in new infections among women of reproductive age (Figure 21).

This result is consistent with PHIA data from several countries showing high treatment coverage in PMTCT target age groups, with more women knowing their HIV status and those who are HIV-positive being on treatment.

Figure 21: Proportion of Pregnant Women Newly Diagnosed Among Those Tested HIV-Positive



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A mother and her baby visit a health clinic in the Dominican Republic.

Women entering pregnancy already on ART have a decreased risk of transmission to the infant – less than 1 percent – compared with up to 25 percent during pregnancy with no treatment. This leads to better outcomes for women, children, and families. PEPFAR's support has been essential for these achievements, and continued focus on PMTCT is needed to both maintain these results and to improve outcomes in countries that continue to have higher mother-to-child transmission rates such as Nigeria and South Sudan.

PEPFAR has invested significantly in PMTCT and provided extensive support for the use of lifelong ART for all HIV-infected pregnant and breastfeeding women, an approach that leads to the best outcomes for women and their partners and children. Since the announcement of the *Global Plan* at the United Nations (UN) in June 2011, the number of new HIV infections in infants each year has dropped by 60 percent in the 21 Global Plan priority countries in sub-Saharan Africa. Following recommendations of WHO's 2015 publication, *Guideline on When to Start Antiretroviral Therapy and on Pre-exposure Prophylaxis for HIV*,[6] PEPFAR has worked to ensure that all supported countries are providing lifelong ART to pregnant women living with HIV. Further, through the *Start Free Stay Free AIDS Free* initiative, PEPFAR and multilateral partners will continue to work toward elimination of mother-to-child transmission by preventing infections in HIV-free young women and identifying and providing treatment to those living with HIV.

PEPFAR supports an effective PMTCT cascade of interventions – antenatal services, HIV testing, and use of ART for life; safe childbirth practices and appropriate breastfeeding; and

[6] WHO. (2015). Guideline on When to Start Antiretroviral Therapy and on Pre-exposure Prophylaxis for HIV.

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infant HIV testing and other postnatal care services – that usually results in an HIV-free baby and a mother with a suppressed viral load. In 2017, PEPFAR continued to ensure that our resources were targeted to high-burden areas to ensure strong linkages for HIV-positive pregnant women to the continuum of care. Antenatal care (ANC) uptake is needed to provide PMTCT services, but rates of ANC uptake differ greatly among communities and countries.

To address these barriers, PEPFAR uses site-specific data to ensure resources are focused in the highest burden areas with the greatest need to maximize the impact on babies and their mothers. In addition, to increase the quality and uptake of ANC in these areas, PEPFAR is working to map areas of low ANC attendance compared with HIV burden and target interventions. The ultimate goal is to achieve ANC attendance for all women and to offer HIV testing to all pregnant women in ANC in our supported areas.

An additional benefit of this site-level analysis is the utilization of program data to geographically map the HIV epidemic at a granular level. This analysis is being replicated across partner countries to further focus the HIV response and understand the evolving epidemic at a geographic and facility level. The addition of recency assays to HIV testing in pregnant women in select DREAMS districts allows for the identification of women with HIV infection acquired in the past six months who are at the highest risk both for transmission to the fetus/infant and to their sexual partners. It also allows mapping of where new infections are occurring for targeting of intensive prevention and testing activities.

PEPFAR continues to focus testing in high-HIV-burden areas. We increased our focus on supporting pregnant women who test negative for HIV to stay HIV-free through increased partner testing and linkages to prevention interventions, including linkage to DREAMS programming and to PrEP for pregnant women at high risk of acquiring HIV. This platform gives PEPFAR the opportunity to engage HIV-negative women in prevention activities and to empower them.

In Kenya, one solution being implemented by a DREAMS Innovation Challenge partner is integrating PrEP provision into maternal and child health and family planning sites and leveraging these platforms to reach HIV-negative women who are at a high risk of infection. This model will be replicated in additional countries in the coming year to increase access to prevention among high-risk young women.

Through partner testing, ANC clinics are key settings to identify serodiscordant couples – when one partner is HIV-positive and the other is HIV-negative – and provide interventions that can lower the risk of HIV transmission, such as immediate treatment for men living with HIV to reduce their transmission risk and referral to VMMC for men who are HIV-negative to reduce their HIV acquisition risk.

PEPFAR's Mozambique team presented FY 2017 Quarter 3 data showing the potential effects of increased partner testing of men through ANC settings, which resulted in more men receiving treatment (Figure 22). Reaching men and identifying those living with HIV is often difficult since asymptomatic men rarely access the health care system. The ANC platform represents an important access point. In the coming year, PEPFAR teams will be introducing self-testing for HIV into ANC to allow women to provide tests for their male partners who may not be able to accompany them to their ANC visits.

In FY 2017, 11.2 million pregnant women learned their HIV status thanks to PEPFAR support. Of those identified as HIV-positive, 94 percent received ARVs during their pregnancy to reduce

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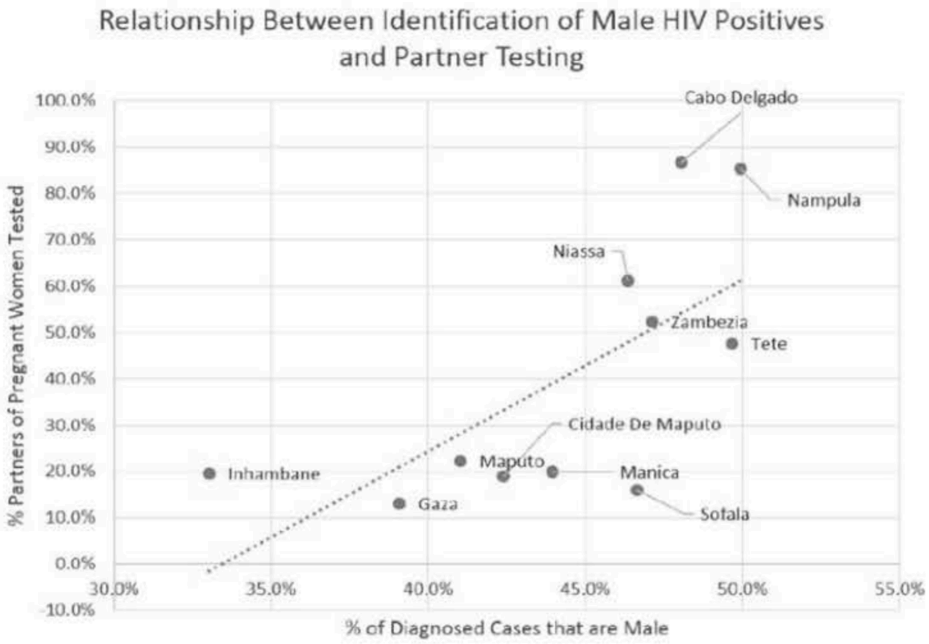
Students participate in a live radio show with Zathu Radio in Malawi.

Figure 22: PEPFAR Mozambique Data from FY 2017, Quarter 3, Showing an Increased Proportion of Men on Treatment for HIV with Increased Partner Testing in the Antenatal Clinic



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A mother with her baby in Zimbabwe.

vertical transmission. ART reduces mother-to-child transmission at birth to less than 5 percent. Transmission rates under 1 percent are seen among women who conceive while on ART and who continue their ART throughout pregnancy with suppressed viral load.

While 95 percent of babies are born HIV-free with effective interventions at birth, if their mothers do not remain on treatment, there is a 15 to 25 percent risk for infection to be transferred to the infant during the breastfeeding period. The breastfeeding period is therefore a crucial time for women to be retained in care and on ART. PEPFAR recognizes the need for data on retention of pregnant and breastfeeding women, and now requires partner countries to report the percentage of women known to be alive and on treatment 12 months after the initiation of lifelong therapy.

In 2017, PEPFAR's retention rate for pregnant women on ART was 70 percent. In some cases, loss to follow-up in one clinic is related to women seeking care at a different clinic after delivery closer to where they stay with their family postpartum. In several countries, public electronic systems and unique identifiers are being developed to allow for better tracking and coordination of care. Increased efforts are being directed at retaining pregnant and breastfeeding women in care and treatment and providing testing for their infants to allow for early treatment of infected infants. For example, in Mozambique, areas with low retention of pregnant and breastfeeding women intensified efforts to provide mentor mothers who support fellow mothers living with HIV and encourage them to take their medicines and attend clinic visits, and monitor retention rates monthly to ensure improvements.

Pregnant and breastfeeding women are priority populations for providing viral load testing to ensure their viral suppression or provide enhanced counseling for ART adherence if they are not yet suppressed. If HIV is suppressed to undetectable levels, the risk of transmission to the fetus during pregnancy, to the infant during breastfeeding, and to sexual partners is essentially zero. PEPFAR advocates for countries to prioritize viral load testing for pregnant and breastfeeding women within their national guidelines. For example, Uganda is updating its national guidelines on testing for viral load to include instructions on more frequent testing for pregnant and breastfeeding women to optimize their treatment. With concerted efforts for optimizing the detection, care, and treatment for pregnant and breastfeeding women living with HIV, transmission to infants can be virtually eliminated. In addition, the ANC platform can be utilized to maximize prevention opportunities to keep young women HIV-free.

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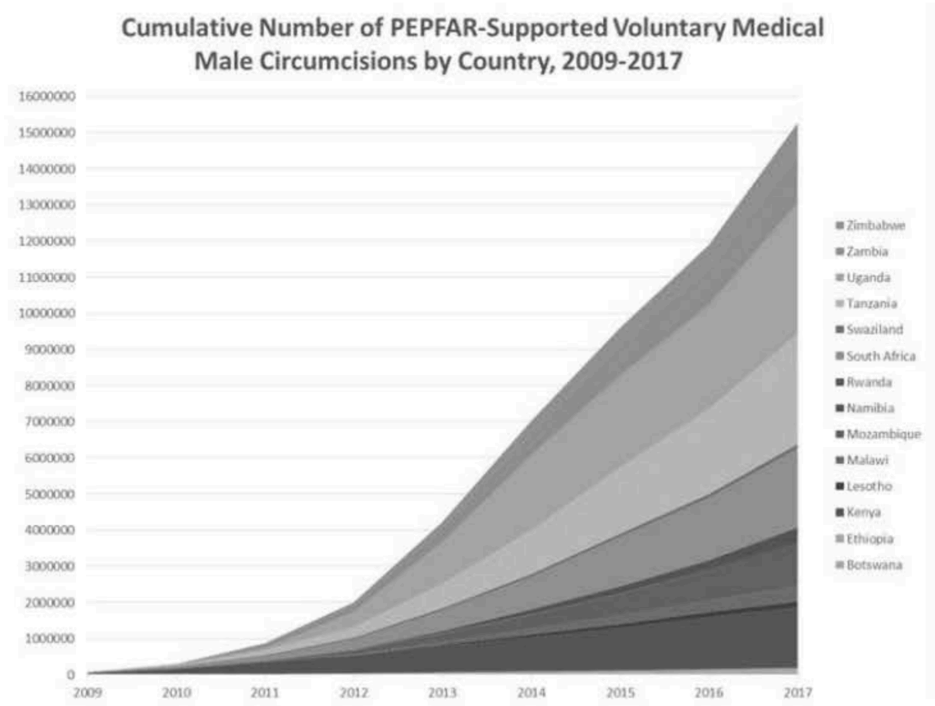
Preventing New HIV Infections in Young Men: Voluntary Medical Male Circumcision (VMMC)

VMMC is a one-time, low-cost intervention shown in randomized controlled trials to reduce men's risk of HIV by approximately 60 percent, with the prevention effect maintained for life. Male circumcision has the potential to prevent millions of new infections, preserve millions of lives, and save billions of dollars in averted HIV treatment costs. Importantly, the procedure brings men, some for the first time since childhood, into health services.

PEPFAR is targeting men ages 15–29 for VMMC to maximize the preventive benefits, with the expanded inclusion of boys ages 10–14 as saturation is reached in the older age groups. Maximum benefit is seen when the circumcision is done before sexual debut, and the most immediate benefits are obtained by focusing on the 15–29 age group. Further, by prioritizing high-HIV-transmission areas among the 14 PEPFAR VMMC priority countries with low background circumcision rates, PEPFAR partners are maximizing efficient and timely implementation to reduce HIV incidence.

As of the end of FY 2017, cumulatively, PEPFAR had supported more than 15.2 million VMMC procedures in eastern and southern African countries, thereby exceeding our ambitious goal, set forward at the 2015 United Nations General Assembly Sustainable Development Summit of 13 million PEPFAR-funded VMMCs, by more than 2 million. PEPFAR supported 3.38 million VMMC procedures in FY 2017 alone, 46 percent in the 15–29 age group (Figure 23). Early modeling suggests that achieving 80 percent coverage of VMMC among males 15–49 years old in the 14 priority countries would prevent

Figure 23: PEPFAR Cumulative VMMC Results, FY 2009–2017



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A group of young boys pose for a photo prior to receiving circumcision at a health clinic in Swaziland.

millions of HIV infections and save billions of dollars. PEPFAR continues to prioritize this one-time intervention by increasing central funding to this intervention in 2018.

Scaling up VMMC to achieve a coverage of at least 80 percent in men ages 15–29 is a key PEPFAR focus and requires continued efforts to improve target setting, demand creation (where appropriate), and efficiency, all of which rely on better site-level data (Figure 24). PEPFAR is implementing innovative solutions to address barriers to VMMC uptake, including through increased staffing capacity and training to meet the annual seasonality of the intervention. In the past year, a PEPFAR-funded tool was shown to effectively optimize site utilization in Mozambique, matching demand for VMMC with staff capacity. Paired with Geographic Information System mapping, this tool led to marked increases in VMMC in the provinces where it was used (Figure 25).

Following the success in select provinces in Mozambique, these methodologies are being shared throughout VMMC programs. In Zimbabwe, aggressive expansion of outreach-based VMMC services, and a hybrid service model where roving full-time VMMC specialists fill short-term personnel gaps and provide capacity-building mentorship at government VMMC sites, resulted in dramatic growth for the PEPFAR VMMC program, culminating in 159,243 circumcisions performed in FY 2016 and an anticipated substantial increase to 227,299 in FY 2017.

Enhanced partner management that included weekly target setting and reporting of results also contributed to the program's enhanced performance. In the summer of 2017, South Africa launched a highly successful acceleration plan to

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Figure 24: Number of Circumcisions by Priority Age Band and Priority Country, FY 2017

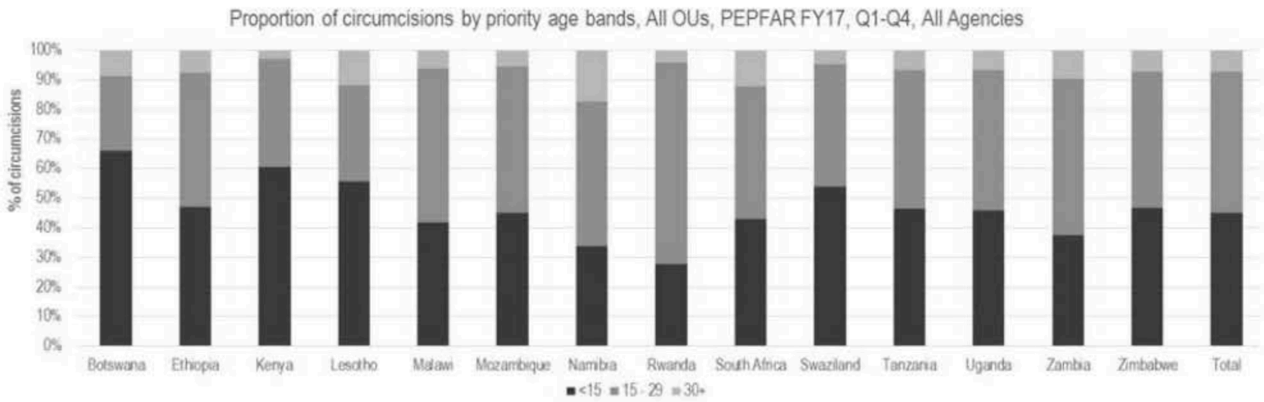
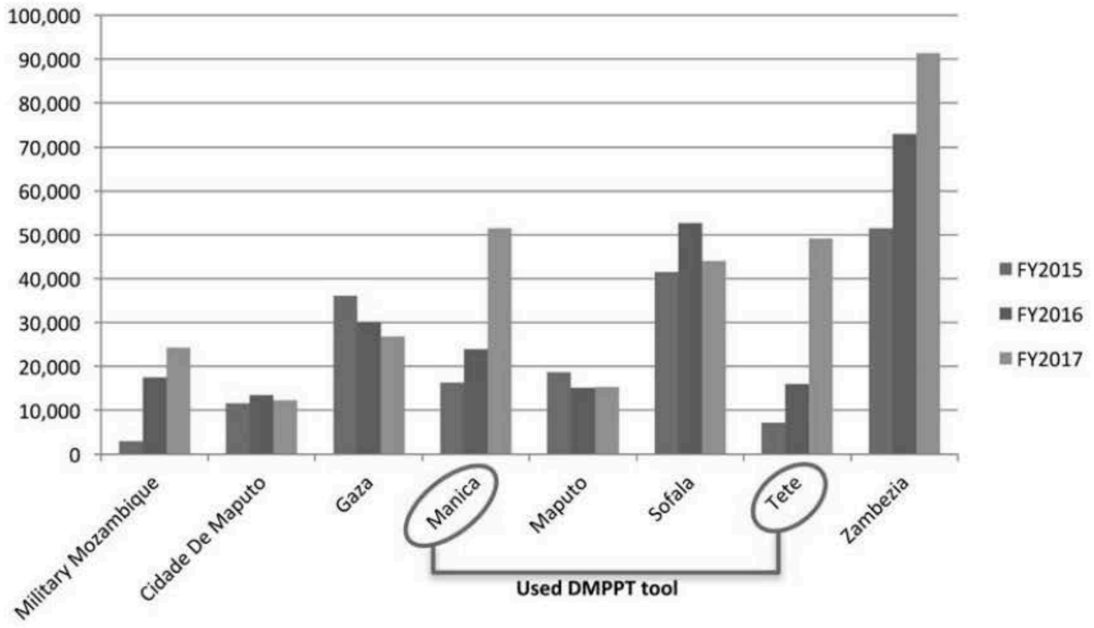


Figure 25: Change in VMMC Numbers in Manica and Tete Provinces in Mozambique Using the DMPPT Tool, Compared with Provinces That Had Not Yet Used the Tool



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rapidly improve VMMC through increased partner management, weekly reporting and monitoring, increased engagement with traditional and community influencers, expanded service delivery, and refined age band targeting. Even in the short term, this campaign resulted in a marked increase in program performance.

Prioritizing Prevention of New HIV Infections in Women, Adolescent Girls, and Children

According to the recent PHIA results, several countries are approaching epidemic control among older adults – Lesotho, Malawi, Swaziland, Zambia, and Zimbabwe. Despite these substantial gains, the PHIAs also reveal key gaps for young women and men under age 35 who are significantly less likely to be tested, on treatment, and/or virally suppressed.

Girls remain up to 14 times more likely to be infected with HIV than boys the same age due to the unique and often inequitable circumstances affecting their daily lives.[7] This is compounded by the rising population of adolescents in sub-Saharan Africa due to the youth bulge and the persistent cycle of HIV transmission between AGYW and young adult men. Now, more than ever, it is evident that there is still much work to do for this population.

The data show that girls and young women account for around three quarters of new HIV infections among adolescents in sub-Saharan Africa.[8] To control the epidemic in this highly vulnerable population, PEPFAR has partnered with the Gates Foundation, Girl Effect, Johnson & Johnson, Gilead Sciences, and ViiV Healthcare on the DREAMS PPP. DREAMS is a comprehensive prevention program addressing the multidimensional circumstances that place young women at increased risk of contracting HIV. DREAMS was launched on World AIDS Day in 2014 and followed by the DREAMS Innovation Challenge in 2016.

The goal of DREAMS is to reduce new HIV infections in AGYW by providing a holistic and layered approach to HIV prevention. DREAMS originally operated in 10 countries that represent more than half of all infections occurring among AGYW globally: Kenya, Lesotho, Malawi, Mozambique, South Africa, Swaziland, Tanzania, Uganda, Zambia, and Zimbabwe.[9]

PEPFAR was pleased to announce exciting new DREAMS results on World AIDS Day 2017. In the 10 African countries (63 districts) implementing DREAMS, the majority (41, or 65 percent) of the highest HIV-burden districts achieved a decline in new diagnoses among AGYW ages 15–24 of more than 25 percent since 2015; 14 of those districts had a decline of greater than 40 percent. Importantly, new diagnoses declined in nearly all DREAMS intervention districts.

Given the impact DREAMS has had on reducing new infections, PEPFAR continues to invest in comprehensive and layered prevention in AGYW through DREAMS incorporation into the PEPFAR COP processes in 2017, thereby institutionalizing the DREAMS core package of interventions as standard PEPFAR programming. In 2017, five additional countries – Botswana, Cote d'Ivoire, Haiti, Namibia, and Rwanda – received funds to implement DREAMS packages with an emphasis on programming focused on preventing sexual violence and HIV through avoiding sexual risk among 9- to 14-year-olds.

[7] ICAP. (2016). Zambia Population-Based HIV Impact Assessment ZAMPHIA 2015–2016. Columbia University, Health MSoP.

[8] UNAIDS. (2015). Empower Young Women And Adolescent Girls: Fast-Tracking The End Of The AIDS Epidemic In Africa. Retrieved from http://www.unaids.org/sites/default/files/media_asset/JC2746_en.pdf

[9] UNAIDS. (2014). The Gap Report: Adolescent Girls and Young Women. Retrieved from http://www.unaids.org/sites/default/files/media_asset/02_Adolescentgirlsandyoungwomen.pdf.

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CREDIT: Girl Effect

Students participate in a live version of the Zathu radio show in Malawi and are given an opportunity to interact with the cast.

DREAMS and Private Sector Engagement

Private sector and philanthropic partners remain central to the success of DREAMS by contributing their unique expertise and capabilities. For example, Girl Effect conducted extensive research to understand the context of relationships between boys and girls in Malawi and what messaging would resonate most with adolescents.

The research found that relationships between boys and girls are highly sexualized in Malawi, and society often discourages boys and girls from being platonic friends, putting girls at greater risk. Girl Effect created a youth brand called *Zathu* (meaning "ours") to close the gender gap through the power of music and storytelling, and tackle challenging topics such as relationships, confidence, and friendships between girls and boys.

Since its launch, *Zathu* has reached more than 1 million people through various platforms. Brand ambassadors have reached 300 youth clubs, more than 7,500 young people, and more than 200 village heads; digital platforms such as the *Zathu* website, YouTube, Facebook, and malawi-music.com have been accessed more than 1.02 million times; and 2,500 text messages have been received from the audience since its launch. Johnson & Johnson has also brought girls' voices to life to tell success stories, inform programming, and amplify DREAMS messaging, and conducted market segmentation analytics to better understand the behaviors of AGYW. Gilead continues to support the purchase and distribution of PrEP medication to AGYW and has supported the registration of PrEP across DREAMS countries, and ViiV Healthcare is investing in capacity building of community-based organizations through the DREAMS Innovation Challenge. The Gates Foundation is supporting

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an impact evaluation as well as implementation science (IS) research to understand program results and evaluate the success of DREAMS.

Implementation of the DREAMS Innovation Challenge began in 2017 to identify innovative solutions that complement the core DREAMS program. Supported by PEPFAR, Johnson & Johnson, and ViiV Healthcare, the Innovation Challenge aims to infuse new thinking and high-impact approaches to meet the needs of AGYW in sub-Saharan Africa, engage new partners that have never received PEPFAR funding, and support funding for small organizations including local, community-based, youth-led organizations.

JSI Research & Training Institute, Inc. (JSI) continues to manage the execution of the Innovation Challenge on behalf of PEPFAR, providing technical assistance, capacity building, and grants management to 45 PEPFAR-funded grantees across all 10 DREAMS countries. Given that nearly half of grantees are new to PEPFAR funding and two-thirds are small community-based organizations, the first year of Innovation Challenge implementation was spent heavily on training, capacity building, start-up activities, and preparing grantees for reporting on program indicators.

In addition, JSI worked on cross-collaboration and coordination in-country among the core DREAMS program and Innovation Challenge key stakeholders. Through the Innovation Challenge, PEPFAR aims to identify new approaches and solutions to addressing the complex needs of AGYW. For example, in Lesotho, Luboto Library Partners created a mentorship program at local libraries where girls can learn about self-esteem, empowerment, and resilience.

After attending the mentoring sessions, one beneficiary stated, "*I am determined to share what I have learned with the girls in Nyimba, especially my sister, who needs to go back to school. I want to teach them about resilience, about getting back up when life knocks you down. I am ready to go home and take a stand.*"

Complex Challenges in the Lives of AGYW

PEPFAR is dedicated to continued implementation of our 2013 Gender Strategy. The Strategy calls for providing gender-equitable HIV prevention, care, treatment, and support; implementing GBV prevention activities and post-GBV care services; implementing interventions to change harmful gender norms and promote positive gender norms; bolstering gender-related policies and laws that increase legal protection; and expanding gender-equitable access to income and productive resources, including education.

In carrying out the Strategy, PEPFAR recognizes the complexities of young women's lives and focuses on holistically addressing factors that

A young woman supporting the DREAMS program in Zimbabwe.

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are related to their HIV risk. Programming includes increasing access to secondary education, reducing GBV, building strong parenting/caregiver relationships, and changing harmful community norms and structures that may make it difficult for young women to navigate their daily life challenges.

For example, evidence shows that remaining in school is a protective factor for adolescent girls. Further, additional years of education reduce the risk of HIV acquisition in adolescent girls and contributes to their future economic success.[10] Each year of secondary school education reduces the risk of child marriage for girls by six percentage points.[11] Education also improves girls' overall health and reduces their number of unplanned pregnancies. Transitioning girls into secondary school and supporting completion of their education has become a high priority for PEPFAR and is supported by both DREAMS and OVC programming.

Violence is another factor placing AGYW at risk, and it permeates the lives of girls and women all over the world. One in three women will experience GBV in her lifetime, increasing the likelihood of contracting HIV and other sexually transmitted infections (STIs).[12] The PEPFAR-funded Violence Against Children (VAC) surveys reveal that, in some countries, one in three girls' first sexual encounter is forced or coerced.[13] PEPFAR is working on reducing violence in the lives of AGYW while also mitigating the impact of violence through education and implementation of post-GBV care. Across DREAMS countries, communities are participating in programs such as Stepping Stones and SASA! that improve outlook on gender norms and reduce instances of violence. PEPFAR recognizes there is a gap in programmatic intensity in preventing the rape of 9–14-year-old girls in communities around the world, and more needs to be done to prevent rape and provide post-rape care. PEPFAR continues to reach tens of thousands of women with post-GBV care, including post-exposure prophylaxis.

Girls often find themselves with very limited economic opportunities. Women make up more than half of the world's population, but only 40 percent of the global labor force.[14] Beyond struggling economies, there are numerous barriers that prevent women from finding jobs or creating businesses, including their level of education and the sexism they often face in hiring. The DREAMS Innovation Challenge is finding new ways to bridge girls to employment.

The complex risks faced by girls often begin when they are very young. That is why PEPFAR is significantly expanding efforts to support adolescent girls ages 9–14 through the prevention of sexual and gender-based violence and helping young people delay their age of sexual debut. Early sexual debut, defined as first sexual intercourse before the age of 15, correlates with multiple risks for girls, such as increased risk of HIV acquisition, substance abuse, school dropout, and early pregnancy.[15, 16, 17, 18] PEPFAR is deploying evidence-based strategies to help adolescent girls avoid risk before it begins.

[10] De Neve et al. (2015). The Lancet.
[11] World Bank and ICRW. (2017, June). Economic Impacts of Child Marriage: Global Synthesis Brief. Washington, DC.
[12] United States Agency for International Development. (2015). Report on Gender Equality and Women's Empowerment.
[13] U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. (2015). Prevalence of Sexual Violence Against Children and Use of Social Services – Seven Countries, 2007–2013 (MMWR Vol. 64, No. 21).
[14] Pew Research Center analysis of national labor force survey data aggregated by the International Labor Organization, accessed March 1, 2017.
[15] UNAIDS. (2016).
[16] Mengo, Small, Sharma, & Paula. (2016).
[17] Tucker-Halpern, Spriggs, Martin, & Kupper. (2009).
[18] Harrison, Cleland, Gouws, & Frolich. (2005).

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DREAMing of an AIDS-Free Future for AGYW

Country ownership of HIV prevention for AGYW is strengthening DREAMS programs and expanding DREAMS-like activities beyond DREAMS districts. The government of Malawi released an AGYW strategy, South Africa started a national program called "She Conquers," and Swaziland expanded DREAMS nationally. The Global Fund, World Bank, and various UN agencies are all increasing their focus on AGYW. DREAMS is not a moment; it is a movement.

Girls are being empowered to step up as leaders in their communities to speak openly about their risks for HIV, and they are embracing programs to mitigate these risks. Parents and caregivers are gaining the necessary skills to engage honestly with their daughters – and sons – about HIV and other issues affecting their health and overall quality of life. Community and faith leaders are dedicating themselves to changing harmful gender norms that increase the vulnerability of girls and young women.

As communities continue to galvanize around AGYW, PEPFAR enthusiastically collaborates and supports evidence-based programming to reduce their risk for HIV and the negative outcomes that increase as girls move into adolescence and young adulthood.

Preventing Stigma and Discrimination

PEPFAR has focused on responding to the significant unmet need for comprehensive prevention, care, and treatment programs and services among key populations globally alongside targeted efforts to support key populations at the local and national level. While strides have been made to strengthen HIV clinical and community services serving key populations, size estimates are frequently inaccurate, and there is a lack of adequate resources invested in programs to address social and structural issues that inhibits access to and retention in quality HIV services. It is essential to address socio-structural factors such as stigma, discrimination, violence, and law enforcement harassment. These factors create barriers to accessing HIV services and limit the effectiveness of service delivery.

In FY 2017, PEPFAR reached more than 7.1 million members of priority populations and more than 2.1 million members of specific key populations with HIV prevention packages (Appendix W). PEPFAR is focused on ensuring that reaching individuals at risk results in a prevention cascade that ensures 100 percent of those reached are tested for HIV and 100 percent receive prevention and treatment services. PEPFAR is funding the Linkages Across the Continuum of HIV Services for Key Populations Affected

A young boy in Mozambique.

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Safe spaces supported by the DREAMS partnership give young women living in informal settlements in Nairobi a place to express themselves without the fear of judgment or harm and learn to stay HIV-free.

by HIV Project (LINKAGES) in an effort to accelerate the ability of partner governments, key population-led civil society organizations (CSOs), and private sector providers to plan, deliver, and optimize comprehensive HIV prevention, care, and treatment services to reduce HIV transmission among key populations and help those who are HIV-positive live longer. LINKAGES enhances HIV prevention and care by improving the outreach to key populations most at risk of acquiring or transmitting HIV, promoting routine HIV testing and counseling, actively enrolling those with HIV into care and support interventions, and enabling them to remain in care.

The project also helps countries to use and scale up evidence-based approaches to service provision; assists key populations to mobilize and advocate for changes in laws and the conduct of police, health care workers (HCW), and policymakers; and works with governments to make programs sustainable for the long term. Other PEPFAR-supported initiatives to reach key populations include the Key Populations Challenge Fund, the Key Populations Implementation Science Initiative, the Local Capacity Initiative (LCI) (Section 4), the Robert Carr Civil Society Networks Fund (RCNF) (Section 4), and a partnership with the Elton John AIDS Foundation (EJAF) (Section 4).

PEPFAR has identified a number of possible solutions to help address some of the barriers to comprehensive prevention, care, and treatment facing key populations. For instance, perceived stigma from HCW, experiences of discrimination while seeking health services, and services that are unresponsive to unique needs are all documented barriers to HIV services, particularly

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for key populations. With the broad adoption of universal Test and Treat in PEPFAR-supported countries, a larger number/proportion of key population clients will be referred for ART initiation and retained in clinical services as countries strive to achieve epidemic control. Persistent stigmatizing and discriminatory treatment of key populations by HCW and others encountered in clinical settings warrant specific measures to improve key populations' access to friendly and competent ART services. As a first step, specific training is required to reduce access barriers by sensitizing HCW to the unique context of key populations. Increasing HCW competency at providing comprehensive, nonstigmatizing services will improve access to services for those with the greatest need and those that are historically underserved.

PEPFAR has also identified enhanced peer outreach approaches and social network strategies as a way to reach increasing numbers of key populations. Key population programs have typically relied on peer outreach workers to reach key population members who frequent hotspots or visit drop-in centers. However, these programs quickly reached testing saturation as the same key population subpopulations received services with frequency. In the quest to improve HIV-positive test yield, several countries piloted and scaled up enhanced peer outreach approaches and social network strategies that take advantage of characteristics of social/sexual/drug-using networks to increase case-finding effectiveness and efficiency.

These newer case-finding approaches were deployed to improve access by and identification of previously untapped KP network members (e.g., KPs who are at higher risk of HIV or who have never been tested) to increase HIV-positive test yield and to find higher absolute numbers

A group of young people in Cambodia take part in a workshop focusing on outreach to key populations.

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A young girl with her caregiver in Uganda.

of HIV-positive key populations for potentially lower investment. Previous research in the U.S. demonstrated greater HIV testing efficiency associated with incentivized approaches that enlist HIV-positive and high-risk HIV-negative individuals to refer their contacts (e.g., friends, sexual partners, drug-using partners, etc.) to HTS.

In addition, PEPFAR has placed a priority on engaging law enforcement in key population programming. Key populations experience high levels of violence all over the world, which increases their HIV risk, decreases their rates of HIV testing and partner disclosure, and reduces their adherence to ART. Police cooperation and protection is essential to reducing violence against key populations and operating successful HIV programs for key populations. In Swaziland, the Dominican Republic, and Kenya, high-level advocacy occurs with police leadership to help HIV programs for key populations operate safely. This is accompanied by training police officers to reduce their stigma and discrimination against key populations, interpret local law, increase their understanding of the link between violence and HIV, instill in them a sense that they are part of curtailing the HIV epidemic, and help them understand the needs of victims of violence and link those victims to the other services they may need.

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ACCELERATING ACCESS TO HIV TREATMENT

HIV treatment is one of the most cost-effective investments that we can make toward controlling the epidemic, both for the health of the person receiving the medication and to prevent their onward transmission of HIV. Science shows that one of the most important factors in the successful treatment of HIV is the early initiation of ART. The sooner that a person living with HIV begins treatment, the more intact and effective their immune system remains, and the faster they can achieve viral suppression, which virtually eliminates their risk of transmitting the virus.

As of September 30, 2017, PEPFAR had supported more than 13.3 million men, women, and children on lifesaving HIV treatment – nearly twice as many as only four years ago. Further, PEPFAR is the world's largest supporter of children living with and affected by HIV/AIDS. Without treatment, 50 percent of HIV-positive children will die before their second birthday, and 80 percent before turning 5 years of age. PEPFAR is expanding access to pediatric treatment. This includes by identifying and addressing key barriers to diagnosing children living with HIV and working with industry to ensure that more child-friendly ART regimens, which are both efficacious and affordable, are being produced. As of September 30, 2017, PEPFAR had supported nearly 1 million children on lifesaving ART.

To expand access to HIV treatment, PEPFAR continues to work closely with the The Global Fund to Fight AIDS, Tuberculosis and Malaria, partner country governments, and others, with a focus on reducing duplication and maximizing impact – so that the most people in need of ART can be reached with all available resources. The following section focuses on how PEPFAR is accelerating access to treatment for those infected with HIV, while working to address the key gaps that remain. These gaps include finding, diagnosing, and treating babies, children, and young adults that are all feeling well, early in HIV infection and when symptom-free. This is our collective challenge, and we are working closely with communities to create the messaging to bring healthy people into the health delivery system. This is key for the diagnosis and treatment of early stage HIV, but also for the creation of the community demand of interacting with the health delivery system to prevent all diseases – also essential for global health security to find and diagnose the next zoonotic event before it spreads.

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Young boys in Ethiopia.

CREDIT: USAID

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Rates of Adherence and Retention

PEPFAR evaluates rates of ART adherence and retention across all supported countries by examining the total number of people on treatment from one year to the next. This analysis determines how many individuals stopped their treatment regimen, were lost to follow-up, or died. Generally, this process involves monitoring a cohort of individuals who were on ART for 12, 24, and 36 months or longer. At the end of FY 2017, PEPFAR maintained a 74 percent retention rate at 12 months on ART by programmatic data. Studies have shown that the majority of clients have moved to new sites and remain on treatment, and this is consistent with the PHIA data that show high rates of viral suppression of those on treatment.

Reviewing country- and community-level retention rates helps PEPFAR treatment programs focus on both geographic and programmatic gaps that, if filled, help individuals who start their treatment to remain on it for life. For example, Côte d'Ivoire's treatment retention rate was 70 percent in 2014. However, after ensuring a consistent stock of drugs and supplies, conducting appropriate clinical staff support training on retention issues, and making improvements in loss-to-follow-up and contact tracing, the program's retention rate increased to 85 percent in 2017. As PEPFAR focuses on achieving the first and second "90" in high-burden areas, adherence and retention remain critical to ensuring that transmission, incidence, and costs decline.

Importantly, the PHIAs showed an impressive overall viral suppression for individuals on HIV treatment, demonstrating a very high level of retention and adherence to treatment and high durability of first-line ARVs. Figure 26 shows rates of viral load suppression among adult PLHIV in each district of Lesotho and each region of Uganda. It is important to note that these rates represent the proportion of all PLHIV who are virally suppressed and are not limited to only those with a known HIV status.

In contrast, Figure 34 shows the proportion of people who are on HIV treatment and are also virally suppressed. The final orange bar on each cascade for Swaziland, Lesotho, Zimbabwe, Malawi, Zambia, and Uganda represents viral suppression among adults on HIV treatment. In this example, Lesotho's suppression rate is 88 percent, which means that of all PLHIV who are currently on HIV treatment, 88 percent are currently virally suppressed. These suppression rates measure adherence to medication and clearly demonstrate that, once people know they are HIV-positive and accessing treatment, they stay on treatment. These results are very encouraging and demonstrate that the key gap is in the diagnosis of HIV infection. Less than half of those under age 25 know they are HIV-positive.

Overall community levels of viral suppression are in the 60 percent range and need to reach 73 percent to achieve the goals of 90-90-90 fast track to epidemic control. The key gap to viral suppression is awareness of HIV infection through HIV testing, as we know that once adult men and women are diagnosed, they access and stay on treatment at high rates. Children and adolescents across PEPFAR have lower rates of viral suppression and PEPFAR is investigating whether this is due to increased viral resistance as a result of single-dose nevirapine for PMTCT historically or adherence issues resulting from stock outs and drug side effects. It is also the reason PEPFAR is working intently with countries and communities to move to the more effective and lower side effects of tenofovir, lamivudine, and dolutegravir (TLD) regimens.

To improve our treatment programs, PEPFAR continues to expand access to viral load

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A group of young girls in Malawi.

testing. Routine viral load monitoring is now recommended by the World Health Organization (WHO) and forms the cornerstone of the third "90" of the Joint United Nations Programme on HIV/AIDS (UNAIDS) 90-90-90 goals. People who are known to have an undetectable viral load can also safely reduce their number of clinic visits and lengthen the duration between pharmacy drug refills – from monthly to quarterly or longer. This both lessens the burden on stable patients and decongests health service delivery sites.

Effective prevention programs, alongside successful HIV treatment and viral suppression in 90 percent of HIV-positive individuals within geographically prioritized areas, will prevent the majority of transmissions and lead to eventual epidemic control. During the 2017 COP/ROP process, all PEPFAR-supported countries with site-level availability of viral load testing, national planning, and use of results to provide differentiated patient care developed plans to scale up viral load testing and monitor progress toward the third "90."

Like treatment coverage, rates of viral load suppression can vary geographically. In its most recent PHIA, Lesotho notes that the overall national rate of viral load suppression is 67.6 percent for all HIV-positive individuals, regardless of their treatment status. However, there are significant geographic differences; in Lesotho, suppression ranges between 59 percent and 72 percent of all HIV-positive people depending on the district.

With high national suppression rates, Lesotho and Swaziland are both well on their way to reach the third "90" goal of having 73 percent of all PLHIV virally suppressed. Critically, among the countries described in Figure 26, the viral load suppression for those on treatment range from 83

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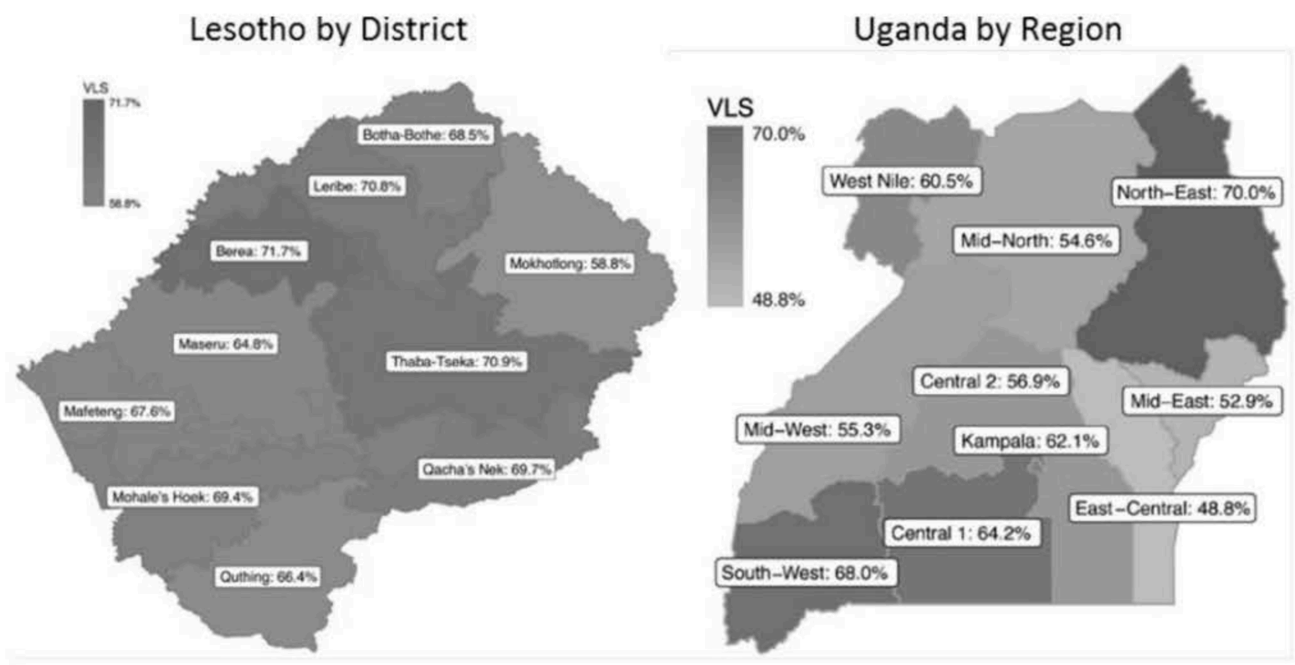
Figure 26: Viral Load Suppression Among HIV-Positive Adults by District in Lesotho and by Region in Uganda



percent to 92 percent, showing high levels of drug effectiveness and individual adherence. Also, the data show that the difference between national viral suppression and those on treatment reflects the gap between those who know their status compared with those who do not, rather than being a function of poor treatment retention.

HIV/AIDS Guidance – New Guidelines: Impact on the Epidemic and Policy Change as Part of Shared Responsibility

The WHO is the leading institution responsible for establishing international normative guidance related to HIV/AIDS programs. In June 2016, WHO released the full, consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection, including PrEP.

In July 2017, the WHO, in collaboration with PEPFAR, the U.S. Centers for Disease Control and Prevention (CDC), and the International AIDS Society (IAS), released key considerations for differentiated ART delivery for specific populations: children, adolescents, pregnant and breastfeeding women, and key populations.

The WHO also released guidelines for managing advanced HIV disease and the rapid initiation of ART, making a strong recommendation for offering ART to PLHIV on the same day that they test positive. By easing the burden on patients and making care more efficient, the program recommendations in these guidelines are helping countries develop more sustainable health care platforms that better foster adherence to lifelong therapy.

The WHO guidelines recommend a reconceptualization of service delivery models to offer more streamlined services to patients

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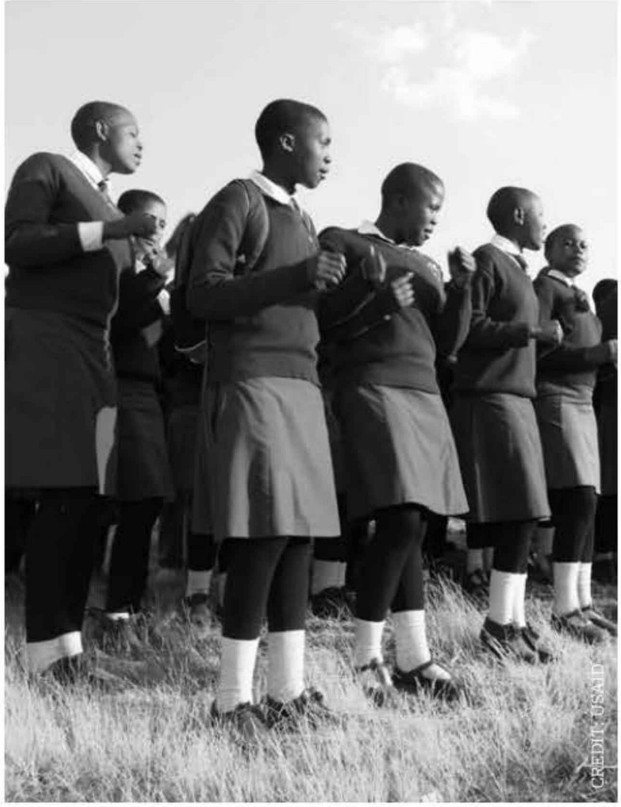
who are clinically well (stable on ART or newly initiating with few symptoms) – estimated to be 80 percent of all those currently on treatment – and more intensive services to those who need it (patients with clinical disease or who are failing therapy). These guidelines also promote more patient-friendly services and expansion of community-based models. As the costs of ARVs declined over the last decade, the costs of ART are now driven largely by service delivery costs rather than drug costs.

Over the last year, PEPFAR has focused on streamlining service delivery and decreasing our costs. Data from Mozambique show that for stable patients, scheduling less frequent clinic visits, prescribing multimonth drug disbursements, and using community adherence groups led to better patient satisfaction and improved adherence, and allowed facilities to treat more patients with existing resources.

The Democratic Republic of the Congo adopted a community-based model, run by community service organizations, through which clients can pick up their medication and receive additional services (such as psychosocial support and counseling) from fellow PLHIV. This model is associated with retention rates of greater than 92 percent in all sites, with most having a retention rate closer to 98 percent. Similar models and others that include community-based drug distribution points are flourishing in other countries.

Through our annual country planning process, PEPFAR has diligently promoted the rapid adoption and implementation of the WHO-recommended Test and Start or Treat All (these terms are synonymous) approach (Figure 28), which reduces the number of patients lost to follow-up, improves the health of PLHIV, and reduces onward transmission.



A group of students in Lesotho.

In South Africa and Kenya, programming for same-day initiation was studied and demonstrated feasibility and success, as well as important obstacles to address. In September 2017, South Africa issued a circular instructing clinicians to provide ART on the same day for PLHIV who are clinically ready and willing to commit to lifelong treatment. In Botswana, Treat All resulted in 76 percent of newly diagnosed PLHIV starting ART on the same day, and an additional 14 percent starting within seven days.

In Tanzania, the Fikia Project outreach to key populations was expanded to 28 districts in FY 2017 and demonstrated same-day linkage to care and ART initiation rates above 75 percent. In Uganda, a coordinated adoption of same-day ART for children under age 15, a hard-to-reach population with historically poor linkage and delayed treatment initiation, resulted in rapidly

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Figure 27: Differentiated Models of Care

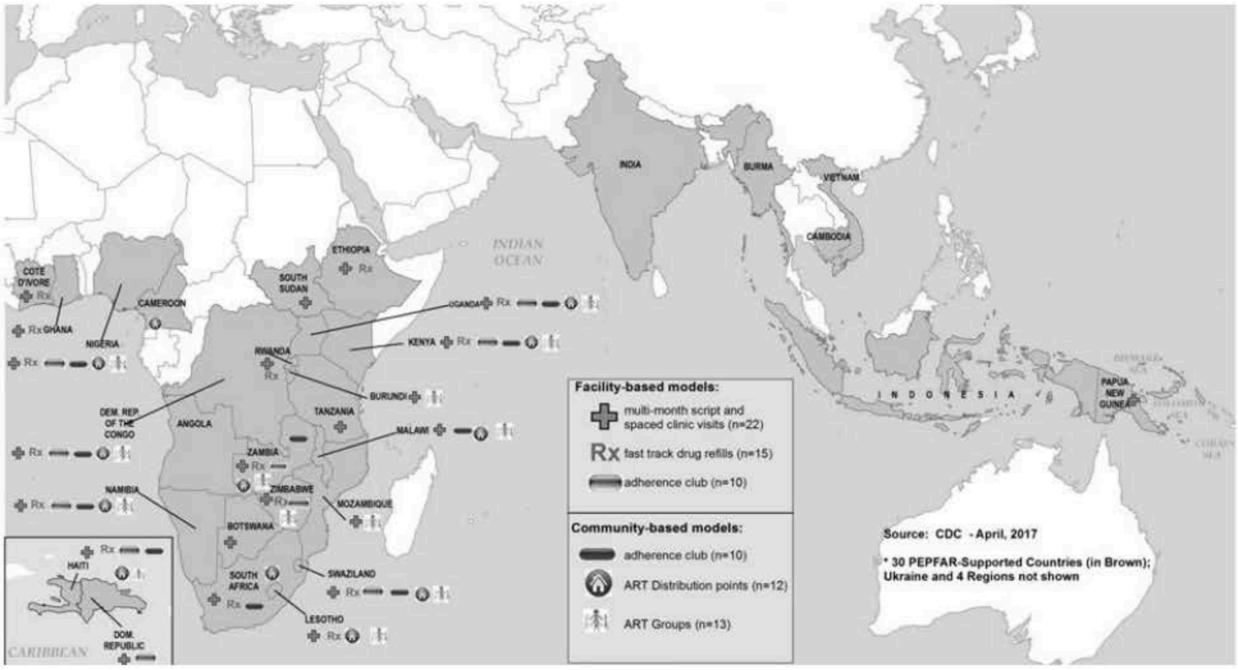
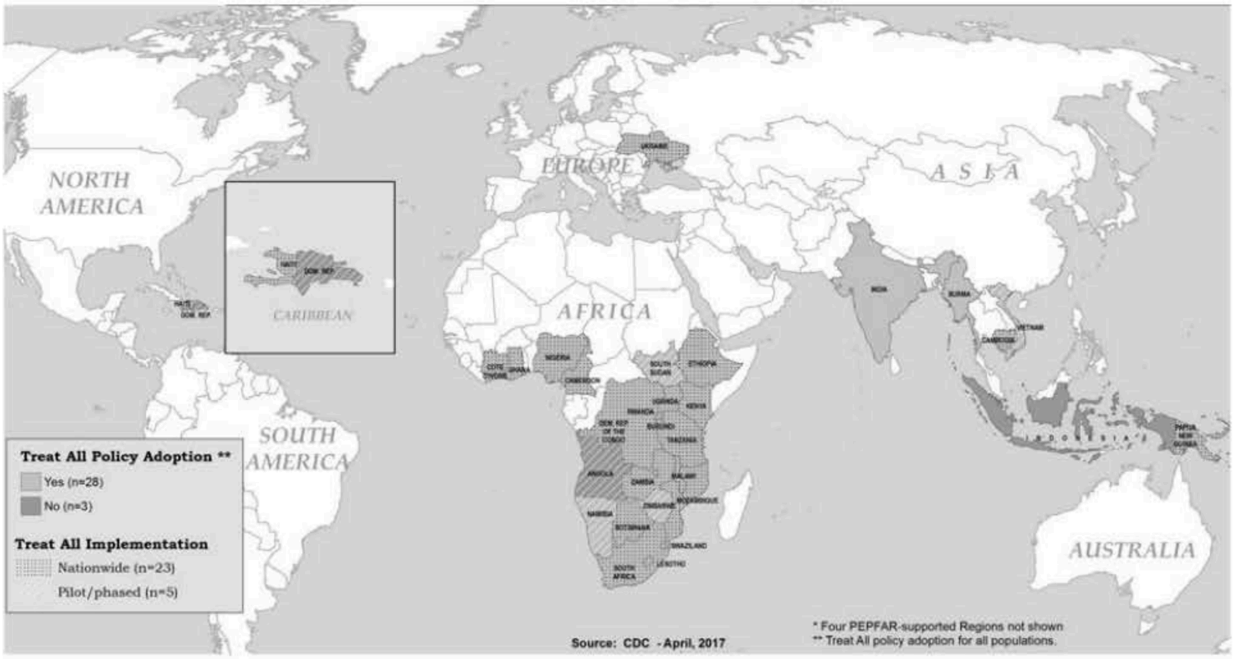


Figure 28: Adoption of WHO Guidelines Recommending a Test and Start (Treat All) Strategy



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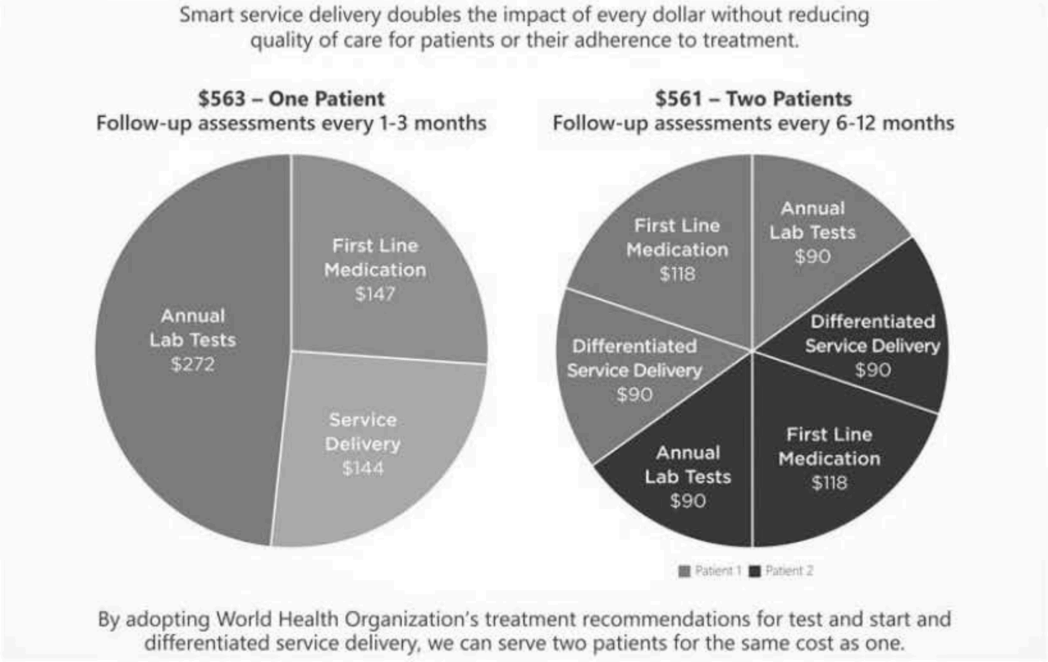
increasing ART coverage among children, now exceeding 67 percent, and a median of zero days between diagnosis and ART initiation.

PEPFAR is committed to taking these insights and clear validation of evidence to scale and ensuring lessons from one country can be adapted to another country without the need to repeat the same studies – ultimately saving PEPFAR and host countries millions of dollars that can be immediately programmed into more effective interventions. However, to be successful, PEPFAR needs to share the study concept during the planning phase, ensuring the study meets the requirements of all of the PEPFAR countries and that research findings can be shared and adapted proactively. PEPFAR has instituted processes to ensure this occurs within the 2018 COP cycle, thereby continuing to adapt management and oversight processes to increase the effectiveness, efficiency, and impact of the program.

The implementation of policy changes is essential to controlling the pandemic. By adopting the WHO's treatment recommendations for Test and Start as well as models of differentiated service delivery, we can serve two patients for the price of one, without reducing either the quality of care for patients or their adherence to treatment (Figure 29). This will expand our impact, save more lives, and avert new infections.

Another key policy adaptation is for VMMC. In September 2016, the WHO released an updated report clarifying the risk of tetanus occurring after circumcision with devices that leave the foreskin in place, compared with surgical circumcision, which immediately removes the foreskin. Data from PEPFAR's VMMC Adverse Event Reporting System were instrumental in determining this risk. The risk of tetanus was estimated to be 30 times higher after device VMMC with foreskin retention compared with surgical VMMC.

Figure 29: Supporting Two Patients for the Price of One



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A mother and her child visit a health clinic in Uganda.

The policy report emphasized the need for two doses of tetanus vaccine before device circumcision unless the client could provide documentation of a full and current tetanus immunization. The report also reiterated the recommendation to provide a single dose of tetanus vaccine before or at the time of surgical VMMC for men without confirmed vaccination status. The WHO report was reviewed and endorsed by the PEPFAR Scientific Advisory Board in November 2016, and PEPFAR continued to work with our partners to scale up effective and appropriate vaccination programming. Again, U.S. ambassadors have been essential in ensuring that this and other critical policy changes occur that work toward epidemic control.

In FY 2017, 3.4 percent of VMMC procedures were performed via circumcision device, and two countries – Rwanda and Zimbabwe – accounted for 92 percent of these procedures. Since Rwanda has a high background level of tetanus immunization, the primary impact of this new policy was expected to be in Zimbabwe. Indeed, the number of device VMMC procedures increased to 93,697 in FY 2017 from 26,721 in FY 2016 in Rwanda, while the number of device VMMC procedures in Zimbabwe dropped to 10,649 from 43,060 in this same period.

In Zimbabwe, from FY 2016 to FY 2017, the new policy helped increase VMMC by 68,056 procedures. In addition, clarification of the risk and policy allowed Uganda to target the two-dose immunization schedule for clients undergoing device VMMC and lift its previous policy of two doses of tetanus immunization for all VMMC procedures. Uganda saw an increase of surgical VMMC procedures to 750,786 in FY 2017 from 321,681 in FY 2016. Thus, PEPFAR data has informed policy, allowing for better client care and greater access to the important preventive benefits of VMMC.

In FY 2017, PEPFAR and other key stakeholders continued to support countries in implementing

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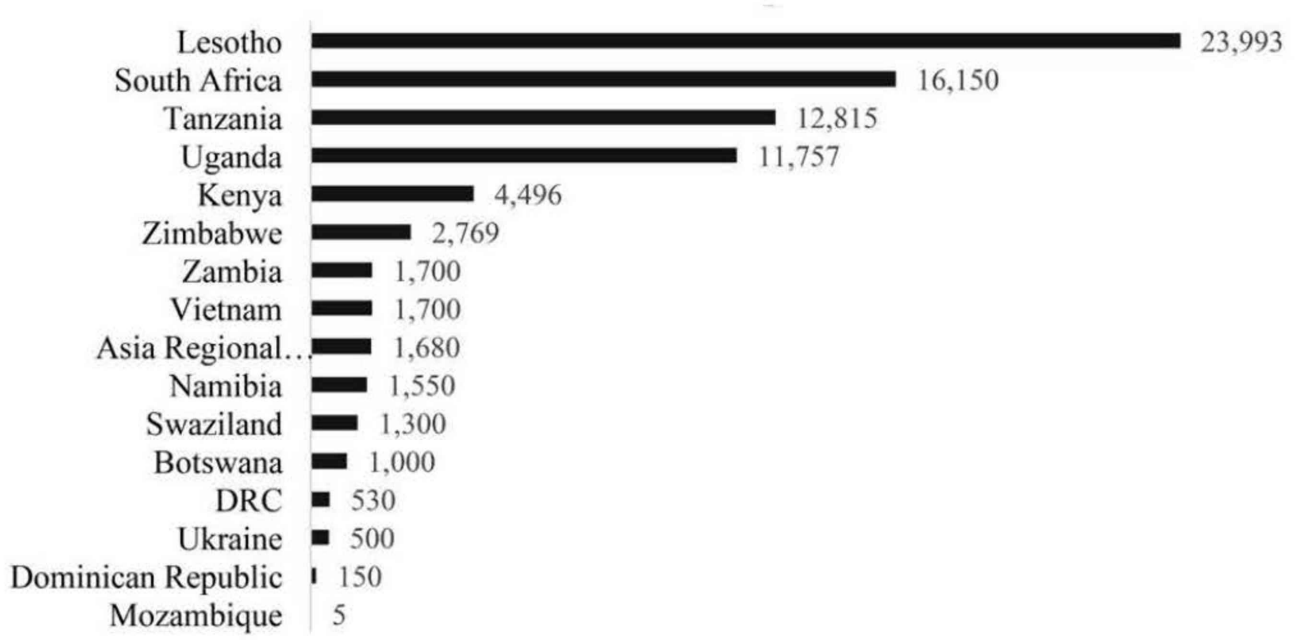
and scaling up PrEP to populations at highest risk of HIV infection. Oral PrEP with oral tenofovir or tenofovir-containing regimens show a reduction in the risk of HIV acquisition among numerous populations.[19]

Since 2015, WHO guidelines have recommended offering oral PrEP to those at substantial risk of HIV infection, defined as an incidence rate of three per 100 persons per year.[20] This level of risk has been seen among serodiscordant couples with inconsistent condom use when the partner living with HIV is not virally suppressed, key populations, and AGYW in many parts of sub-Saharan Africa.

PEPFAR supports WHO guidelines on the use of PrEP as a component of a package of comprehensive prevention services that includes risk reduction education and counseling, condom promotion and provision, VMMC, and structural interventions to reduce vulnerability to HIV infection. In July 2017, the WHO released new implementation tools for PrEP, consisting of 11 modules designed to address above-site and implementations issues. These modules include specific guidance for PrEP users, providers in clinic- and community-based settings, and national authorities.[21]

These new WHO tools were created to support and improve the continued rollout of PrEP. Implementation of PrEP in low- and middle-income countries outside of research studies and demonstration projects has been relatively limited. Accelerating PrEP implementation was a priority for PEPFAR in FY 2017 and continues to be in FY 2018 (Figure 30). PEPFAR supported 10,733 individuals newly on PrEP in FY 2017 across 14 countries. PEPFAR is engaging in PPPs

Figure 30: The FY 2018 Targets for the Annual Number of PEPFAR-Supported Individuals Newly Enrolled on PrEP



[19] http://www.who.int/hiv/pub/arv/arv-2016/en/
[20] http://www.who.int/hiv/pub/guidelines/earlyrelease-arv/en/
[21] http://www.who.int/hiv/pub/prep/prep-implementation-tool/en/

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A famly in Malawi.

to roll out the provision of PrEP. For example, Gilead is purchasing medication and paying operational expenses for the procurement, transportation, and dissemination of PrEP for young women who are uninfected but at substantial risk for acquiring HIV.

PEPFAR in-country teams and implementing partners expanded PrEP provision in multiple countries in FY 2017. An example of success with PrEP implementation in FY 2017 was the PEPFAR-funded, key populations-focused LINKAGES model in Thailand. With this model, PrEP was provided by working with well-established networks of community-based organizations that gave access and support to eligible clients.

PEPFAR is providing updated guidance for FY 2018 on accelerating PrEP implementation through data-driven approaches to identifying prioritized groups, target setting, budgeting, and implementing PrEP as a key intervention for ultimately achieving epidemic control. PEPFAR teams are being advised to work with partner governments to advance above-site PrEP readiness and implementation.

These teams will work to help develop national PrEP policies, adopt implementation and operational guidelines product registration, support awareness-building and demand-creation efforts, test integrated PrEP service delivery models, and explore private sector engagement. Given that PrEP rollout is still in its early phase, PEPFAR recommends that in-country teams work with established key or other vulnerable populations.

HIV Burden and Treatment Response

At the end of 2016, there were 36.7 million PLHIV globally, including nearly 23.6 million

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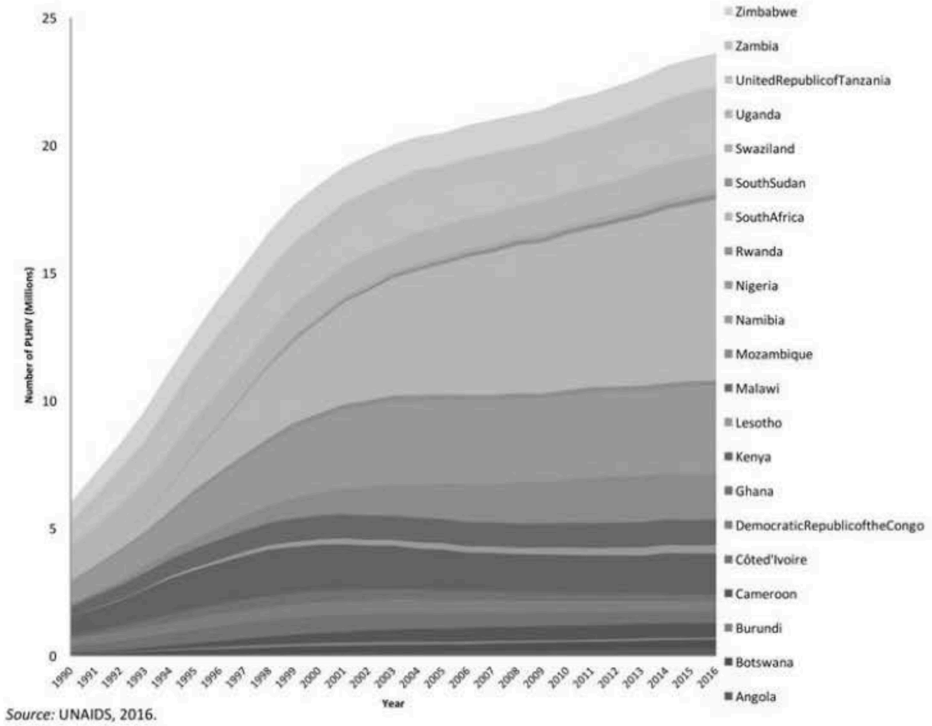
in sub-Saharan Africa (Figure 31). As treatment programs are implemented across partner countries, PLHIV are able to live longer and more productive lives. It is clear from the graphic that the most rapid increase in PLHIV is in South Africa. Controlling the pandemic will require a new approach to South Africa in partnerships with communities and the host government. PEPFAR is working closely with South Africa as they have dramatically increased their own resources committed to their HIV response, have aligned resources with the highest burden areas where transmission continues unabated, and expanded prevention efforts. The South African government funds 80 percent of the HIV response, but more is needed now to change the course of this pandemic. In other countries where the specific depth and breadth of the epidemic is less known, PEPFAR will use the PHIA data from Nigeria, Kenya, Haiti, and Rwanda to validate the Spectrum Model and define future investments to ensure the epidemic is being addressed in the most effective and focused manner for impact. In another series of countries, including most of west and west central Africa, Tanzania, Mozambique, and South Sudan, the majority of citizens are unaware of their status and need more effective testing and expansion of treatment to change the course of the HIV pandemic. Country-by-country data allow PEPFAR, host-country governments, and communities to program resources in the most effective and impactful manner.

Globally, the number of people on HIV treatment and lives saved increased from 2003 to 2016, largely due to the contributions of PEPFAR and the Global Fund, working closely with partner countries. In the large majority of countries, the expansion of treatment was slow but steady from 2004 to 2007 (PEPFAR Phase I), after which enrollments on treatment increased. From 2008 to 2010 (beginning of PEPFAR Phase II), enrollments rapidly increased and continued along similar trajectories. In 2014, PEPFAR

Figure 31: Cumulative Trends of National Data, Persons Living with HIV, Sub-Saharan Africa: 1990–2016



Source: UNAIDS, 2016.

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A group of teenagers in Nicaragua.

partnered with countries to refocus efforts to high-burden areas and started monitoring the epidemic at the community level, accelerating progress with sustainable results. From 2015 to the current reporting period, enrollment increased even more rapidly, in a revenue-neutral manner, as programs increase efficiency and focus on moving toward the goal of epidemic control.

There has been a dramatic increase in people receiving ART since 2004, as shown for PEPFAR-supported countries in Africa in Figure 32. There was a flattening of the treatment expansion in the 2013–2014 timeframe in most countries, but that recovered in 2014–2016 with the realignment of resources to the U.S. congressional 50 percent care and treatment earmark, and the program closely tracking both the slope of scale-up of services and the geographic coverage. This tracking helps ensure countries are reaching at least 80 percent treatment coverage at the subnational level while community viral load levels are suppressed to undetectable. It is clear that both the speed to reach greater service coverage and the percentage coverage are important to controlling the HIV epidemic. The commitment to monitor treatment coverage saves lives and decreases transmission. PEPFAR-supported countries made significant progress in reaching UNAIDS 90-90-90 targets, with five countries approaching epidemic control (Figure 34).

The rapid implementation of evidenced-based interventions has been a primary driver of the dramatic declines in new HIV infections and mortality rates. Ongoing success toward controlling the HIV/AIDS epidemic is completely dependent on continuing and accelerating this momentum. Far fewer individuals under age 25 know their HIV status, are on treatment, or are virally suppressed as compared with older adults. Combined with the doubling of the population aged 15–24 in sub-Saharan Africa, the HIV pandemic could

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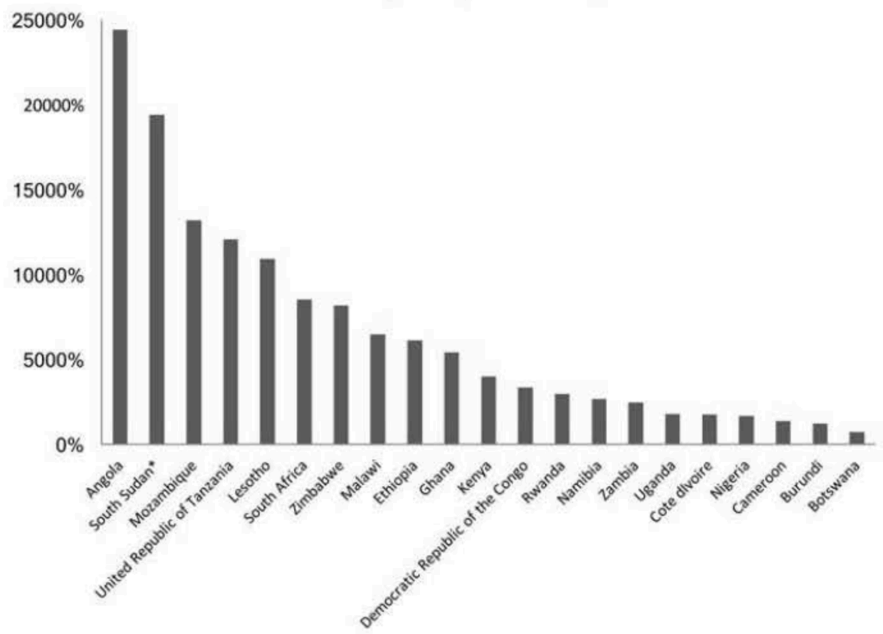
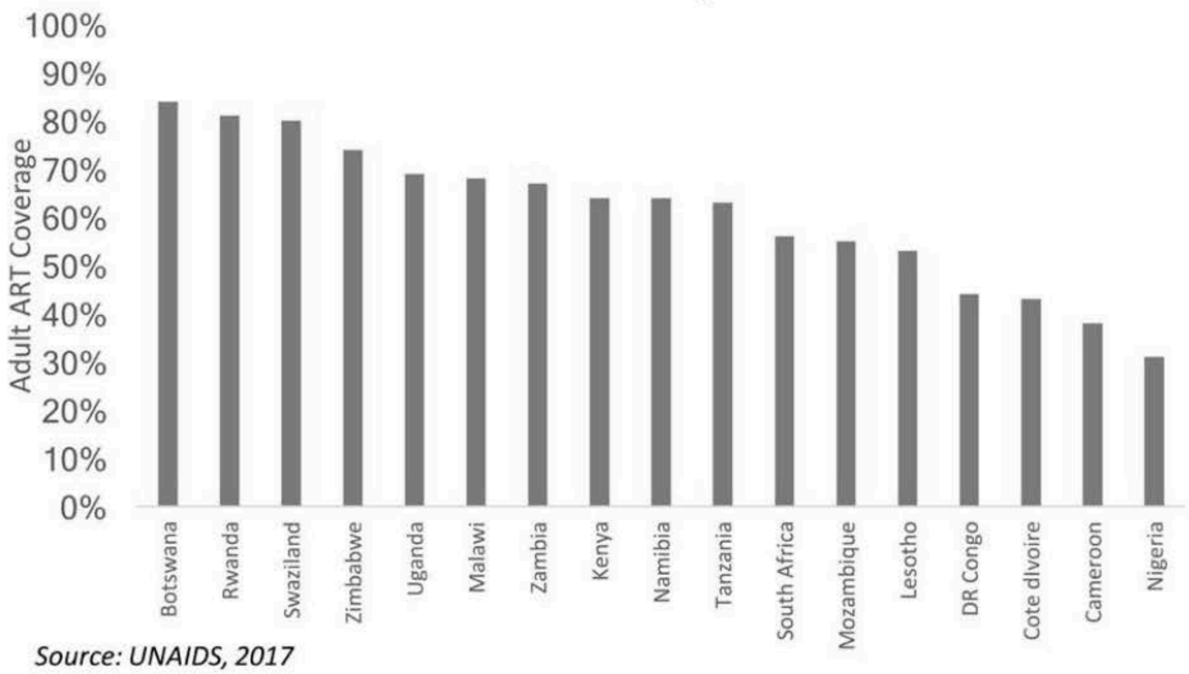
Figure 32: Host Country National Data, Percent Increase of People HIV+, Adults (15+), Receiving ART (2004–2016), Select PEPFAR-Supported Countries in Africa, 2016



Source: UNAIDS, 2016.

Figure 33: Adult (15+) Antiretroviral Treatment Coverage, Select High-Burden Countries, 2016



Source: UNAIDS, 2017

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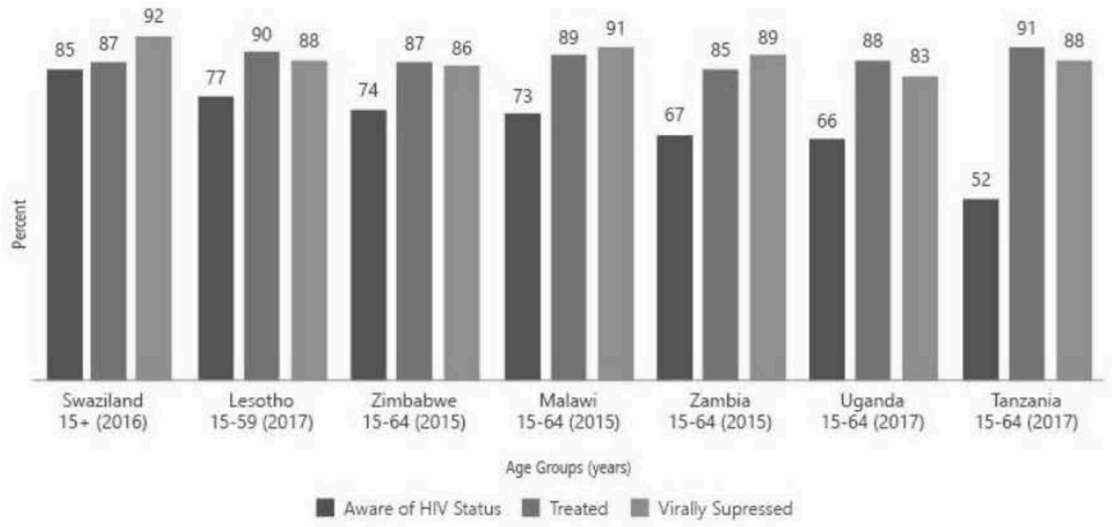
Figure 34: PHIA Data Showing Progress Toward 90-90-90 Targets in Ages 15–64 in Lesotho, Malawi, Swaziland, Tanzania, Uganda, Zambia, and Zimbabwe



dramatically expand without concentrated and concerted efforts to reach this age group. PEPFAR will continue to focus intensely on 15–24-year-old AGYW through increased prevention efforts, including the following:

- Continuing to expand effective components of DREAMS programming. Impact data from the PEPFAR-led DREAMS partnership was released in December 2017, and they show a 25–40 percent or greater decline in new HIV diagnoses among AGYW in the majority (65 percent) of the highest HIV-burden communities or districts receiving DREAMS interventions.
- Scaling up VMMC to achieve coverage of at least 80 percent in men ages 15–29; in FY 2017, PEPFAR supported VMMC for nearly 1.6 million men in this prioritized age range.
- Improving HTS to find more men and link them to treatment. PEPFAR supported HTS for more than 85.5 million people in FY 2017 and continued to roll out and scale up innovative testing approaches, including HIV self-testing, index patient testing, and partner testing.

ART coverage rates combine the figures for persons on treatment and those who need ART (as modeled by countries and UNAIDS as all persons with HIV infection). These rates provide a telling story of progress in each country (Appendix W). Some indications suggest that countries with an HIV prevalence of greater than 5 percent are improving at a slightly accelerated rate. However, considerable variation exists across countries. This reaffirms PEPFAR's strategy of utilizing our resources to support services in settings with the greatest need and potential for impact. This strategic focus remains a priority to ensure that countries can aggressively address their epidemics with available global HIV/AIDS funding. Viral loads must be suppressed to control the epidemic and allow communities and countries to thrive.

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One of the more important milestones toward controlling the epidemic is when the annual number of new enrollments in treatment approaches 80 percent at the national level. This transition point reflects a care and treatment scale-up rate that is successfully limiting the transmission of HIV to uninfected persons. A lower number of new infections suggests that the future influx of patients requiring treatment will be more manageable, smaller, and less expensive – causing the epidemic to contract.

This shift in trends, while important in the ongoing effort to control the epidemic, does not imply that continuing efforts can slow down. Any faltering of national treatment efforts may return the trend lines to an earlier, more negative pattern, once again driving up new HIV infections. Any drop in adherence or retention will result in increasing viral loads and substantial surges in HIV transmission.

Supportive Care

In February 2014, PEPFAR developed a strategy for the prioritization of care and support interventions based on an extensive, in-depth review of evidence and best practices. Four universally applicable activities were identified as priorities for the greatest impact on reducing AIDS-related morbidity and mortality:

- Regular clinical and viral load monitoring
- Screening and treatment for active TB and prophylaxis for those without active TB
- Cotrimoxazole prophylaxis for opportunistic infections per PEPFAR country/regional guidelines
- Clinical and nonclinical evidence-based interventions to optimize retention and adherence, including PLHIV community support groups

A group of children in South Sudan.

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A peer health educator in Uganda.

Based on the evolution of WHO guidelines to recommend treatment for all PLHIV, care and treatment were collapsed into one entity, consistent with the guidelines. As a result, additional focus will be placed on strategies to improve linkages, adherence to and retention of care and treatment, viral load monitoring, diagnosis and treatment of TB co-infection, and preventing TB reactivation.

Ensuring Pediatric Treatment and Psychosocial Support So Children Can Thrive

Over the last several years there has been a dramatic decline in new pediatric infections, but children born infected with HIV are still in critical need of lifesaving HIV treatment. In 2016, 2.1 million children under age 15 were living with HIV/AIDS – nearly 90 percent of whom live in sub-Saharan Africa – and one new pediatric HIV infection occurred approximately every three minutes. Without ART, 50 percent of children living with HIV/AIDS will die before their second birthday, and 80 percent will die before their fifth birthday. In 2016, only 43 percent of children living with HIV/AIDS had access to treatment. In west and central African countries, only one in five children living with HIV infection received ART. This must change. Saving the lives of children with HIV is not only the right thing to do; it is the smart thing to do. By treating children early in their HIV infection, they can stay healthy and thrive. Healthy children who can pursue their dreams will grow economies, create jobs, and strengthen their communities for decades to come.

In August 2014, PEPFAR, through the U.S. Department of State, announced the Accelerating Children's HIV/AIDS Treatment (ACT) initiative at the U.S. African Leaders Summit. ACT was a

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two-year initiative to significantly increase the total number of children receiving lifesaving ART in nine high-priority countries in sub-Saharan Africa. The nine ACT countries (Cameroon, Democratic Republic of the Congo, Kenya, Lesotho, Malawi, Mozambique, Tanzania, Zambia, and Zimbabwe) include countries with some of the greatest need for pediatric treatment and some of the greatest disparities in treatment coverage for children compared with adults living with HIV/AIDS. The $200 million initiative represented a joint investment by PEPFAR and the Children's Investment Fund Foundation.

As of the end of September 2017, PEPFAR had supported more than 494,000 children (<20 years old) with lifesaving ART in the nine ACT countries (Table 2). At the same time, across all of the countries for which PEPFAR provides support to children with HIV, nearly 1 million children were receiving ART, demonstrating the impact of our investment far beyond the nine original ACT countries, while more children are thriving and aging out of the pediatric age cohorts and fewer children are being born. Pediatric ART coverage increased by 10 percent or more from 2014 to 2015 in several of the ACT countries (Kenya, Lesotho, Malawi, Tanzania, Zambia, and Zimbabwe), with Zimbabwe reaching 80 percent pediatric ART coverage. We have an excellent road map for how to increase diagnosis and treatment, and this must be translated to scale through all implementing partners across all regions, countries, and counties to ensure all children thrive with HIV.

ACT shows the power of PPPs, and that focus can lead to tremendous impact in just two years. Countries will continue to receive technical support to build on pediatric treatment scale-up in FY 2018. While we increased children's treatment across all ACT countries, we also nearly doubled the number of children on treatment across all PEPFAR-supported countries from 2014–2017.

Adoption of the WHO guidelines to treat all HIV-infected children and adolescents was a critical step in linking HIV-positive children to the care they need and will be a major factor in

Table 2: Accelerating Children's HIV/AIDS Treatment (ACT) Initiative – Children Receiving ART by September 2017

Countries Participating in Accelerating Children on HIV/AIDS Treatment (ACT) Initiative	0–19-year-olds
Cameroon	9,763
Democratic Republic of the Congo	6,534
Kenya	118,665
Lesotho	10, 726
Malawi	34,425
Mozambique	95,844
Tanzania	75,813
Zambia	60,248
Zimbabwe	82,723
All ACT Countries	494,741

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A mother with her baby in Swaziland.

furthering the successes in pediatric treatment attained under ACT. PEPFAR expanded the OVC program to ensure that all vulnerable children have access to HTS, care, and treatment.

PEPFAR's response to OVC continues to evolve in response to changes in the epidemic. While the rate of orphaning continues to decline with the expansion of treatment (Figure 35), significant risks and vulnerabilities remain for children and adolescents as a result of HIV/AIDS. PEPFAR's OVC program serves children in a range of adverse situations, including children who are living with HIV, living with caregivers who are HIV-positive, orphaned, at risk of becoming infected, or a combination of these factors.

Although children of all ages are affected, those at either end of the age 0–17 spectrum face specific heightened risks related to HIV. For orphans specifically, the proportion of children orphaned within each age band rises as children grow older (Figure 36), resulting in a much higher proportion of children in older age bands who are likely to have lost one or both parents.

For the youngest age band (age 0–4) the risks of HIV infection and orphaning diminished greatly due to the expansion of PMTCT and adult treatment. Remaining risks pertinent to OVC programs include loss to follow-up of HIV-exposed infants and suboptimal viral load suppression in children. Additionally, concerns remain regarding potential cognitive delays in HIV-infected infants and, to a lesser degree but still salient, in HIV-exposed but uninfected infants.[22]

The OVC platform's wide network of faith- and community-based staff and volunteers support treatment adherence and proper nutrition for infants and young children, and also provide

[22] Wedi, et al. (2016). Perinatal Outcomes Associated with Maternal HIV Infection: A Systematic Review and Meta-analysis. The Lancet, 1, e33–e48.

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Figure 35: Children Orphaned by AIDS (1990–2016)

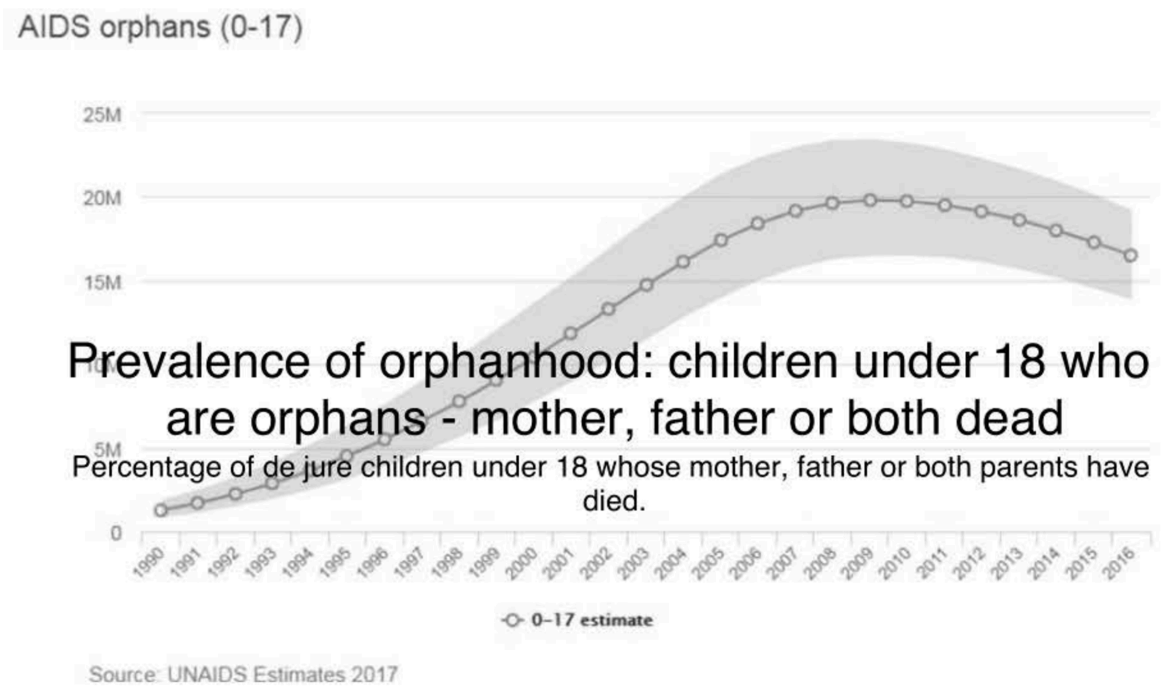
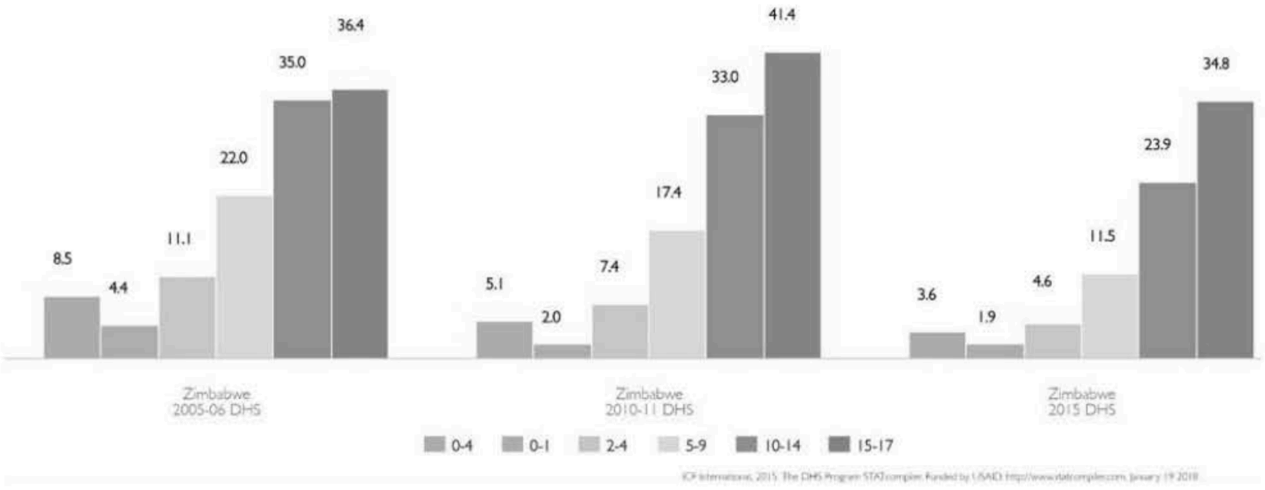


Figure 36: Prevalence of Orphanhood: Children Under 18 Who Are Orphans – Mother, Father, or Both Dead



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A group of students in Ethiopia.

family services such as socioeconomic assistance. For first-time mothers, especially adolescent girls, OVC program case management services that link young mothers to assistance are critical to ensuring that both parent and child remain healthy and AIDS-free. OVC programs are also incorporating activities that promote cognitive stimulation and development into PMTCT and pediatric HIV platforms to help identify and mitigate cognitive effects.

OVC community networks are also helpful in finding older asymptomatic children who are infected with HIV, but whose lack of routine contact with health centers makes them less likely to be diagnosed through traditional clinic-based HIV testing modalities. The ACT initiative significantly increased the number of children on treatment who were more easily identified, but children who are not visibly sick and are past immunization benchmarks are more difficult to find. PEPFAR initiated a new indicator in 2017 that measures the ability of OVC programs to facilitate diagnosis of asymptomatic beneficiaries and ensure that all beneficiaries – newly and previously diagnosed with HIV infection – are receiving lifesaving ART.

Many more children are now on treatment, thanks to the ACT initiative, but viral suppression among young children and adolescents remains a challenge. PEPFAR's OVC partners are working to improve children's treatment outcomes by providing home visits and accompanying children to clinics, and addressing the broader socioeconomic needs of families through interventions such as savings and internal lending groups.

Adolescents living with HIV infection also benefit from the added socioeconomic support available through the PEPFAR-supported OVC platform. Adolescents on ART in South Africa, for example, who had access to multicomponent interventions,

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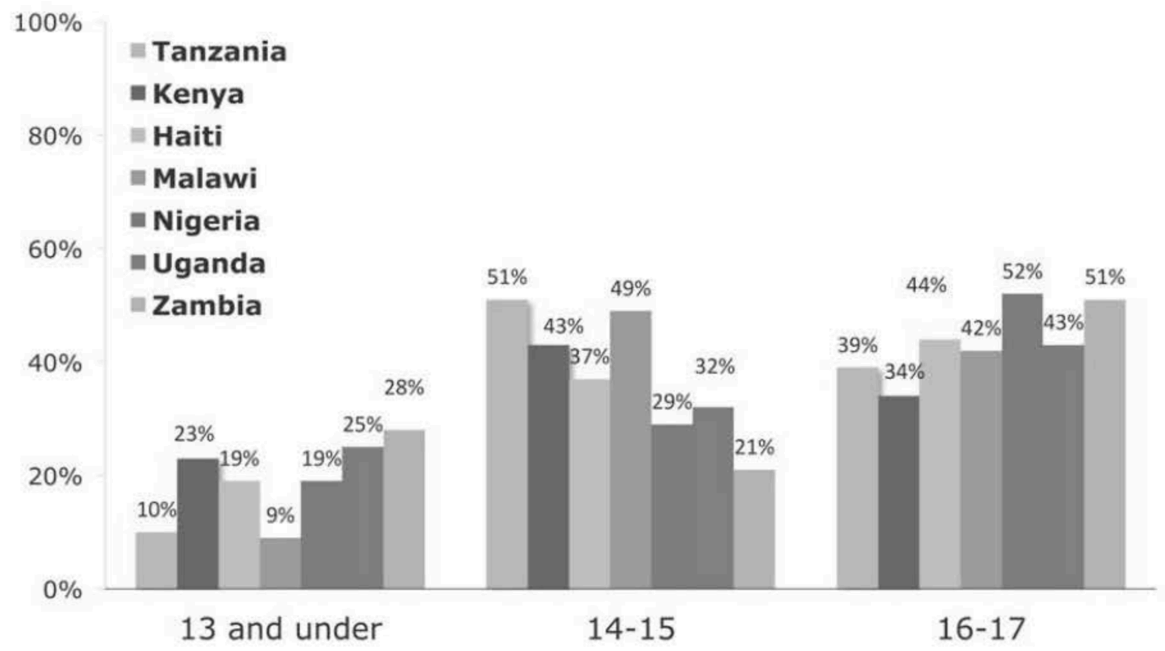
Figure 37: Age at First Incident of Forced or Coerced Sex in Childhood Reported by 18–24-Year-Old Females[25]



including parental monitoring, support groups, and social transfers such as cash and food provisions, had greater adherence than those who did not.[23] For the OVC platform, the focus for adolescents is two-fold: adhering to treatment and living a productive, healthy, AIDS-free life.

As children become young adults, their risk for acquiring HIV through sexual transmission increases sharply. OVC programs are uniquely poised to address the myriad of factors that put adolescents at risk. Adolescent female orphans, for example, have an earlier sexual debut than their nonorphaned (and orphaned) male counterparts.[24] Furthermore, adolescent females orphaned or living with a caregiver who is ill due to HIV have higher rates of transactional or other unsafe sex and higher exposure to physical 5and emotional abuse. VAC surveys in multiple PEPFAR countries show that forced and coerced sex among females can occur at very young ages. In FY 2017, PEPFAR supported activities related to the VAC surveys in six countries.

Since adolescent girls in sub-Saharan Africa are nearly three times more likely than boys to become infected with HIV, OVC programs also serve as a platform for focused efforts, such as DREAMS, that provide an array of protective interventions (e.g., schooling, economic support, parenting, and GBV services).

In FY 2017, PEPFAR allocated $50 million of OVC Plus Up funds to improve the delivery and quality of a comprehensive service package of health and social services. These funds

[23] Cluver, et al. (2016). Achieving Equity in HIV-treatment Outcomes: Can Social Protection Improve Adolescent ART-adherence in South Africa? AIDS Care, 28(sup2), 73–82.

[24] Robertson, et al. (2010). Sexual Risk among Orphaned Adolescents: Is Country-Level HIV Prevalence an Important Factor? AIDS Care, 22(8), 927–938.

[25] https://www.cdc.gov/violenceprevention/vacs/publications.html

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Young girls advocate for access to HIV services in Uganda.

were used to address the unique needs and risk factors of currently served OVC and their families in nine PEPFAR countries – Cameroon, Cote d'Ivoire, Ethiopia, Kenya, Lesotho, South Africa, Tanzania, Uganda, and Zimbabwe.

OVC's access to multiple services demonstrates a compounding effect, yielding greater improvements in health and well-being than isolated interventions. OVC Plus Up fund activities addressed the priority areas of secondary school for adolescent girls, supporting children living with HIV to access and be retained on treatment, GBV prevention and post-violence care, and overall economic strengthening of OVC families in the PEPFAR program. These funds were also used to implement OVC interventions in the areas of primary education, case management, health promotion, early childhood development, parenting, food and nutrition, and psychosocial support.

Although the vast majority of OVC impacted by the AIDS pandemic live with their immediate or extended family members, PEPFAR has also supported evidence-based practice for children living outside of family care. PEPFAR has expanded implementation of the Social Service Systems Strengthening Monitoring and Evaluation Framework to support the everyday functioning of government ministries mandated to prevent and respond to VAC and to prevent separation and promote permanency. In Kenya, for example, PEPFAR, through the U.S. Agency for International Development (USAID), supported the Department of Children Services to roll out a new management information system to improve tracking and monitoring of services delivery. In addition to providing on-site technical assistance to social welfare ministries, PEPFAR has continued to support cross-country experience sharing and evidence-based practice through the Better Care Network, a global learning hub for those preventing and addressing children's separation from family. PEPFAR also worked collaboratively on the Action Plan for Children in Adversity with the U.S. government's Displaced Children and Orphan's Fund and other U.S. government agencies.

PEPFAR will continue to work with OVC implementing partners to ensure that the most vulnerable, at-risk children receive appropriate HIV testing and access to lifesaving services. PEPFAR regularly evaluates OVC programs to ensure they adapt to the changing demographics of the epidemic and the shifting evidence for core interventions. PEPFAR sets aside 10 percent of the bilateral program funding to address the diverse, complex, and often critical needs of OVC. In FY 2017 alone, PEPFAR supported critical care and support for nearly 6.4 million OVC and their caregivers, including nearly 4.8 million children and adolescents under 18 years of age, in order to mitigate the physical, emotional, and economic impact of HIV/AIDS on children. Furthermore, there are 2 million children who did not become

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orphans thanks to PEPFAR-supported treatment of their parents and caregivers living with HIV.

Addressing Tuberculosis/ HIV Co-Infection

TB-HIV Co-Infection

Worldwide, TB is the leading cause of death from an infectious disease, and by far the leading cause of death among PLHIV in sub-Saharan Africa. In 2016, an estimated 10.4 million people developed TB, 10 percent of whom were PLHIV. Of the PLHIV who developed TB, 74 percent were in Africa. In that same year, approximately 1.7 million people died from TB, including 374,000 PLHIV. TB still accounted for more than a third of the estimated 1 million AIDS-related deaths in 2016.

The tragedy is that these deaths are almost entirely avoidable and should no longer be occurring with this frequency. We can prevent cases of TB in the first place by scaling up ART, which reduces the risk that a person living with HIV will develop TB by around 65 percent, and the addition of TB preventive therapy to ART reduces the risk by 97 percent. Among PLHIV who do develop TB, early ART can halve the mortality rate, which is why testing for HIV and initiating ART are key interventions. Globally, however, only 57 percent of notified TB cases have a documented HIV status. Among those with documented HIV infection, 85 percent were started on ART.

Over the past few years, there has been an increase in the provision of TB preventive therapy to persons newly enrolling in HIV care, driven largely by the programmatic activities of PEPFAR-supported countries. In 2016, an estimated 42 percent of new enrollees initiated TB preventive therapy. Yet, if we are to directly address the leading cause of mortality in PLHIV and achieve the goals described in the WHO's End TB Strategy, we need to do better.

A patient receives a TB test in Thailand.

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CREDIT: USAID

A family visits a TB clinic in Kenya.

PEPFAR remains committed to addressing TB as a top policy and programmatic priority. Utilizing the full array of prevention approaches, active TB and HIV case-finding, and access to early treatment, PEPFAR has been instrumental in reducing the breadth and mortality of the TB/HIV epidemic. The continued focus on effective and innovative TB/HIV programming will help consolidate those achievements and further global efforts to end the TB pandemic. In PEPFAR-supported countries in the fourth quarter of FY 2017, the proportion of TB patients with documented HIV status exceeded 95 percent, and the proportion of those with HIV infection who were started on ART was more than 90 percent. PEPFAR recently revised the suite of TB indicators and now requires reporting on initiation and completion of TB preventive therapy as well as TB screening and initiation of TB therapy in those diagnosed with the disease. These additions were part of a deliberate attempt to drive programming and encourage fully integrated TB/HIV care in PEPFAR countries. Encouragingly, despite no requirements for targets or advanced planning, 17 countries initiated or expanded TB preventive therapy programming in FY 2017.

A number of markedly successful programs have been introduced throughout PEPFAR. Mozambique, South Africa, and Zimbabwe all had steady progress incorporating TB preventive therapy into routine care with approaches that are well documented and replicable. More notably, Kenya had tremendous rapid success rolling out TB preventive therapy through a well-designed, carefully crafted collaboration between the country's national TB and HIV programs. Their approach resulted in an increase in the number of PLHIV who initiated TB preventive therapy from a baseline of 10,000 PLHIV in 2014 to more than 490,000 by 2016, with a 90 percent treatment completion rate.

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In Swaziland, a pilot project featuring patient-centered care demonstrated remarkable adherence and completion rates for TB preventive therapy, and cost-effectiveness analyses were modeled for Botswana, India, Tanzania, and elsewhere, setting the stage for committed programming. Historically, in most settings, patients with TB and HIV have been required to visit the two clinics separately, imposing a large burden on patients and compromising care. This is beginning to change: In Swaziland's public clinics, TB/HIV activities are now fully integrated and colocated and boast a 97 percent documentation of HIV status among TB patients and 99 percent ART initiation within six months of TB treatment initiation among those with HIV infection. Kenya is introducing a policy to integrate treatment of HIV into TB clinic visits for the duration of treatment for their TB disease, greatly diminishing the burden on the patients. All of these programmatic approaches are being shared throughout PEPFAR.

PEPFAR continues efforts to support scale-up of the Cepheid GeneXpert MTB/RIF test, a fully automated, molecular diagnostic test for TB. This test enables programs to diagnose TB quickly, which can help reduce transmission and decrease mortality. Even more sensitive cartridges are coming to market, and truly portable devices, which will allow testing at any point of care, will be available in 2018. An inexpensive urine dipstick test for very ill PLHIV presenting late to care was shown to reduce mortality in the hospital setting. PEPFAR will continue to support the expansion of these diagnostic technologies to help ensure that effective TB diagnostic testing is accessible to all PLHIV.

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LEVERAGING PARTNERSHIPS FOR SUSTAINABILITY

PEPFAR forges strategic public-private partnerships that support and specifically complement our prevention, care, and treatment work addressing key gaps in innovative ways. These partnerships translate new ideas into practice and accelerate impact, benefiting from the private sector's expertise and resources as well as its leadership, energy, and inspiration.

PEPFAR also advances global health diplomacy through close engagement with U.S. chiefs of mission globally and with the diplomatic corps in Washington, D.C., as well as by connecting health impacts to other U.S. foreign policy priorities, including economic growth, trade, education, and political stability, to ultimately increase the impact of U.S. health investments and partnerships.

Additionally, since its founding, PEPFAR has built health infrastructure and strengthened capacity through an emphasis on sustainability. We have invested in robust laboratories and well-trained laboratory specialists critical to well-functioning health systems, enabling clinicians and health workers to better diagnose and treat a range of diseases and conditions. PEPFAR has trained nearly 250,000 HCW to deliver HIV care and other health services.

No single actor alone can control and ultimately end the HIV/AIDS pandemic. We need all sectors and diverse partners working together to provide financing, demonstrate political will, and carry out interventions both within and outside of the health sector, and we must always include people directly affected by HIV in any response. We must also continue to act decisively and strategically with our resources and continue to bring other donors and countries themselves to the table to respond to and ultimately help end the epidemic. The following section focuses on how PEPFAR is leveraging our platform and partnerships for sustainability and to accelerate progress toward achieving epidemic control.

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Young girls in Mozambique.

CREDIT: Peace Corps

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Building a Safer, More Secure World Through Strategic PEPFAR Health Investments

PEPFAR's investments are not only controlling the HIV/AIDS pandemic, but are also contributing to stability and American national security. The U.S. government, including through PEPFAR, has built health infrastructure and strengthened capacity with an emphasis on sustainability. This not only has supported patients living with HIV/AIDS, but also has been leveraged to address maternal and child health, TB, malaria, immunizations, and noncommunicable diseases. Since 2015, S/GAC has required the development and monitoring of clear metrics and milestones in the Country Operational Plans (COPs) of all horizontal health system investments, captured in the COPs as Table 6 (described in detail later). This is in addition to the vertical system strengthening investments captured through site-level and above-site-level investments.

In 2017 alone, through PEPFAR's COPs/ROPs (Figure 38), we invested nearly $600 million in horizontal, above-site health system strengthening investments. This includes nearly $100 million to strengthen laboratory systems. The health system strengthening component, specifically laboratory system strengthening, is horizontal in this category supporting the overall system. PEPFAR invests an additional $20–30 million through our Headquarters Operating Plans (HOP). These efforts have also strengthened the ability of countries with sizable HIV/AIDS burdens to swiftly address other outbreaks, such as Ebola, avian flu, and cholera, ultimately enhancing global health security and protecting America's borders.

PEPFAR's support of robust surveillance and health information systems, as well as laboratories and well-trained laboratory specialists critical to well-functioning health systems, enable clinicians and health workers to better diagnose and treat a range of diseases and conditions. PEPFAR has trained nearly 250,000 HCW to deliver HIV care and other health services, improving

Figure 38: Country Operational Plan Investment in Health Systems

OU	Total Table 6	Surveys, studies, pilots, etc.	HRH: Training	HRH: Other HRH Activities	Governance: Test and Start Policy or Guidelines	Governance: Other Governance Activities	Finance	Supply Chain	Inst & Org Development : Civil Society Strengthening	Inst & Org Development : Other I&O Activities	Strategic Information Systems	Laboratory Systems	Pilots	Other
Botswana	$ 10,329,360	$ 2,454,812	$ -	$ -	$ 400,000	$ 536,730	$ 50,000	$ 1,000,000	$ -	$ 238,778	$ 4,310,271	$ 1,338,769	$ -	$ -
Burundi	$ 2,470,000	$ 150,000	$ 200,000	$ 70,000	$ -	$ 525,000	$ -	$ -	$ -	$ 700,000	$ 700,000	$ 125,000	$ -	$ -
Cameroon	$ 4,338,429	$ 250,000	$ 259,174	$ -	$ -	$ 108,857	$ -	$ 1,518,856	$ 38,000	$ 473,510	$ 608,372	$ 1,016,660	$ 65,000	$ -
Cote d'Ivoire	$ 17,446,679	$ 980,000	$ 1,279,000	$ 925,000	$ -	$ 1,116,500	$ 309,000	$ 3,237,177	$ 50,000	$ 2,037,002	$ 2,386,000	$ 3,937,000	$ 350,000	$ 840,000
DRC	$ 10,588,577	$ 750,000	$ 1,125,000	$ 150,000	$ -	$ 255,000	$ -	$ 1,972,827	$ 13,500	$ -	$ 2,372,000	$ 1,204,500	$ -	$ 1,295,750
Ethiopia	$ 25,867,864	$ 1,720,937	$ 3,580,328	$ 714,358	$ -	$ 1,280,486	$ 50,000	$ 1,400,000	$ 888,638	$ 5,354,803	$ 1,804,426	$ 4,339,968	$ -	$ 4,733,920
Haiti	$ 14,610,500	$ 2,595,000	$ 970,000	$ 345,000	$ -	$ 2,770,500	$ 100,000	$ 200,000	$ -	$ 1,500,000	$ 4,430,000	$ 1,700,000	$ -	$ -
Kenya	$ 73,522,446	$ 11,360,000	$ 3,505,525	$ 3,115,000	$ 2,170,000	$ 7,198,637	$ 800,000	$ 1,000,000	$ -	$ 3,000,000	$ 19,300,000	$ 15,662,130	$ -	$ 6,411,154
Lesotho	$ 10,129,873	$ 3,000,000	$ 100,000	$ 189,873	$ -	$ 200,000	$ -	$ 1,500,000	$ -	$ 450,000	$ 4,130,000	$ 260,000	$ -	$ -
Malawi	$ 20,936,791	$ 4,230,000	$ 2,709,650	$ 4,292,883	$ -	$ 1,171,335	$ -	$ 2,791,650	$ 667,503	$ 800,000	$ 552,975	$ 1,865,000	$ 400,000	$ 1,455,795
Mozambique	$ 36,978,367	$ 3,640,402	$ 2,665,004	$ 2,823,046	$ 180,000	$ 3,322,625	$ -	$ 5,061,145	$ 91,143	$ 1,412,381	$ 11,794,580	$ 5,289,684	$ 698,357	$ -
Namibia	$ 7,906,799	$ 1,420,000	$ 1,387,701	$ 267,730	$ -	$ 732,164	$ 300,000	$ 664,000	$ -	$ 261,000	$ 1,316,115	$ 583,089	$ -	$ 975,000
Nigeria	$ 46,060,928	$ 15,039,806	$ -	$ -	$ -	$ -	$ 1,800,000	$ 3,770,500	$ 100,000	$ 3,119,400	$ 9,149,400	$ 11,131,373	$ 750,449	$ 1,200,000
Rwanda	$ 8,733,833	$ 3,358,050	$ 1,143,041	$ 350,900	$ -	$ 348,823	$ -	$ 621,504	$ 19,000	$ 458,114	$ 1,257,023	$ 741,040	$ -	$ 436,338
South Africa	$ 71,271,514	$ 7,302,915	$ 4,654,114	$ 8,921,766	$ 1,179,685	$ 8,597,903	$ 1,804,473	$ 6,989,970	$ -	$ 17,580,791	$ 5,700,000	$ 2,606,625	$ -	$ 5,933,272
South Sudan	$ 8,373,350	$ 1,860,000	$ 50,000	$ 200,000	$ -	$ 1,203,350	$ -	$ 530,000	$ 800,000	$ 450,000	$ 1,075,000	$ 1,200,000	$ -	$ 1,005,000
Swaziland	$ 7,370,730	$ 380,000	$ 95,000	$ 2,300,500	$ -	$ 920,000	$ 13,000	$ 600,000	$ 350,000	$ 650,000	$ 1,750,000	$ 85,000	$ -	$ 227,230
Tanzania	$ 44,522,000	$ 2,900,000	$ 1,613,000	$ 8,403,000	$ 1,090,000	$ 6,160,000	$ 2,950,000	$ 2,590,000	$ 1,700,000	$ 200,000	$ 10,366,000	$ 3,700,000	$ -	$ 2,850,000
Uganda	$ 43,079,968	$ 3,801,200	$ 420,000	$ 11,149,118	$ -	$ 6,484,825	$ -	$ 5,243,616	$ 294,000	$ -	$ 11,341,806	$ 4,345,403	$ -	$ -
Ukraine	$ 10,389,928	$ 1,014,575	$ 585,000	$ 900,000	$ 490,000	$ 1,690,236	$ 595,000	$ 600,000	$ 200,000	$ 2,165,000	$ 765,000	$ 840,000	$ -	$ 545,117
Vietnam	$ 12,123,121	$ 450,100	$ 1,422,300	$ 401,700	$ 130,000	$ 3,012,928	$ 1,257,000	$ 654,400	$ 604,345	$ 253,000	$ 2,002,788	$ 1,143,000	$ 7,800	$ 783,760
Zambia	$ 81,186,034	$ 2,810,000	$ 4,888,202	$ 11,252,516	$ -	$ 869,735	$ 158,752	$ 20,938,150	$ -	$ 805,906	$ 13,875,663	$ 25,587,110	$ -	$ -
Zimbabwe	$ 19,314,356	$ 750,000	$ 542,689	$ 1,450,000	$ -	$ 2,518,000	$ -	$ 6,964,159	$ 347,700	$ -	$ 1,862,199	$ 3,886,703	$ -	$ 992,906
TOTAL	$587,551,447	$ 72,217,797	$33,194,728	$ 58,222,390	$ 5,639,685	$ 51,023,634	$10,187,225	$ 69,847,954	$ 6,163,829	$41,909,685	$112,849,618	$ 92,588,054	$ 2,271,606	$ 29,685,242

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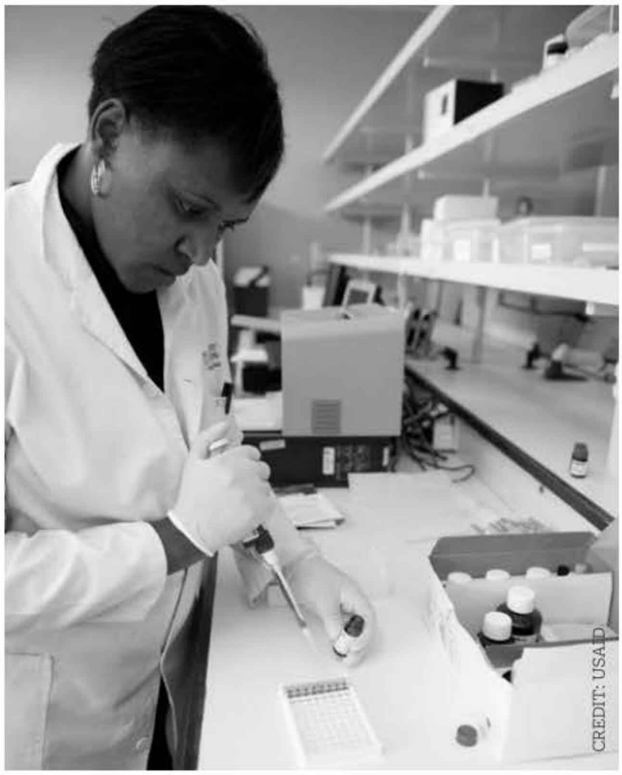
HIV care and creating a lasting infrastructure that enables partner countries to address the health challenges of today – and tomorrow.

The value of the American people's investments in PEPFAR also goes beyond health. A 2015 study by the Bipartisan Policy Center shows that, from 2004 to 2013, political instability and violence fell by 40 percent in countries that received PEPFAR assistance versus only 3 percent in similar countries that did not. The strength of the rule of law increased 31 percent versus only 7 percent.

PEPFAR has also helped advance economic development, particularly in sub-Saharan Africa, by extending not just average life expectancy, but the quality of that life, and the ability to engage in labor. That has a ripple effect, as far fewer parents, siblings, and children die of AIDS – or need to leave school or work to take care of family members dying from AIDS. All of which support economic growth and enlarge potential markets for American goods and services.

These critical health systems investments have enabled PEPFAR to directly support the global health security agenda (Figure 39). The programs that PEPFAR supports are about delivering results today, but also about creating lasting services, systems, and policies that will allow countries to be resilient and successful tomorrow. Healthy populations are a prerequisite for prosperous and stable societies. Investing in the well-being of our partner countries not only saves lives, but also enhances global public health and security. In our interconnected world, there is no better investment. Americans at home and abroad are safer and more secure when systems to prevent, detect, and respond to infectious disease threats are strong.

As demonstrated during the West Africa Ebola outbreak in 2014, in our interconnected world, infectious diseases are only a short flight away. PEPFAR has not invested heavily in countries like Liberia, Sierra Leone, and Guinea due to their low HIV prevalence. As such, their health systems were not equipped to detect the Ebola outbreak earlier when it could have been contained, preventing risk to American aid workers in-country and its ultimate spread to those within the U.S. In contrast, thanks to the capacity built, in large part with U.S. support through PEPFAR, in countries like Nigeria, DRC, and Uganda, when the Ebola crisis hit, they were able to contain it, preventing its spread to their neighbors and international travelers.



A lab in Kenya.

Above all else, the backbone of PEPFAR and the key to our success toward controlling the HIV pandemic and contributing to a sustainable health system is the interagency partnership – a whole of U.S. government approach. PEPFAR was established with a commitment to program resources and leverage competencies differently. PEPFAR harnesses the unique capacities in each U.S. government agency to create a

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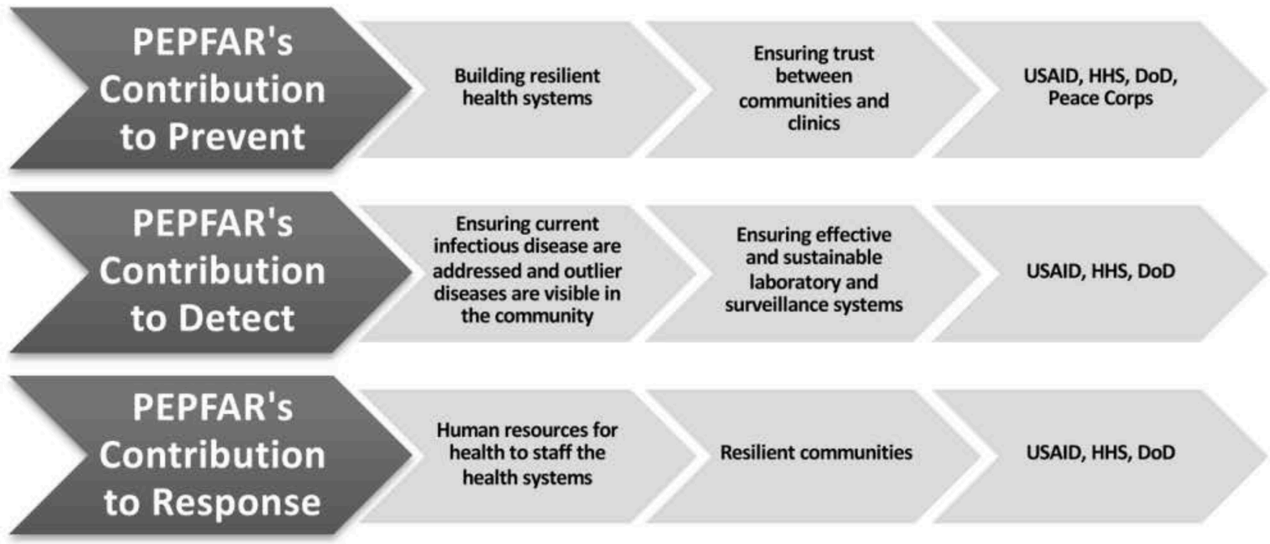
Figure 39: PEPFAR's Contribution to Global Health Security Agenda



comprehensive and highly efficient approach to programmatic impact coordinated by the Department of State. PEPFAR models the key whole-of-sector and horizontal integration of line ministries that are required by host governments to successfully control the HIV pandemic and build sustainable health systems.

Sustainability of these efforts and ending AIDS as a public health threat cannot be accomplished by any single actor. It requires all sectors working together to contribute financing, demonstrate political will, and carry out interventions both within and outside of the health sector. Partnerships enable business to enter new markets; gain exposure to new customers; enhance brand and goodwill in communities; recruit, retain, and engage the workforce; ensure the health and wellness of employees; and contribute to the global HIV/AIDS response.

PEPFAR has strategically partnered across multiple sectors, including with the private sector, and is increasingly looking to its business expertise to help solve global health challenges. We are translating the optimized business practices to our global health world to improve the efficiency of our programs.

Driving a Sustainability Agenda with Country Partners

The global response to the HIV pandemic is unprecedented. Billions of dollars and millions of people have been mobilized to save lives and accelerate progress toward controlling the pandemic. Together, we have achieved remarkable gains. If country governments, donors, and civil society work in partnership and continue to ensure every investment has a clear outcome, it is within our grasp to reach epidemic control. Yet, our progress is at risk if we do not take decisive actions to ensure the HIV

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response is sustainable. We must take concrete actions to increase our impact and accelerate our progress toward long-term sustainability.

Given the magnitude of our contributions to the global HIV response, PEPFAR plays a major role in determining the future path of the HIV epidemic and bears great responsibility for ensuring that the HIV response is sustainable. Indeed, all PEPFAR investments move us closer to sustainability; only an epidemic that is shrinking and not expanding is financially sustainable.

Ultimately, PEPFAR's achievements will be measured by our contribution to sustained control of the HIV epidemic. However, PEPFAR is not in this alone and all HIV development partners must do their part. As a key element of our partnerships with country programs, PEPFAR needs every country to make the health systems investments required for ensuring sustainability through increased national resources allocation and mutual accountability for results.

Table 6: Operationalizing Sustainability

PEPFAR is deeply committed to firmly embedding sustainability principles and programming into our annual COPs. The major innovation in this direction in 2016 was the Table 6 process. As part of the Strategic Direction Summary (SDS) that is included within each COP, PEPFAR added a new requirement for country teams to assess the major policy and systemic gaps that inhibit attainment of 90-90-90 treatment goals and longer term programmatic sustainability. These gaps are analyzed and distilled in Table 6 of the SDS. In 2016, there was special emphasis on addressing barriers to Test and Start policies and barriers to implementing differentiated care.

A group of children in Cambodia.

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A group of young girls in South Africa.

The purpose of Table 6 is to present multiyear efforts that contain several components or activities grouped together with indicators that show annual progress toward a longer term goal above the site of service delivery. In essence, Table 6 brings future end-state goals into present-day budgeting and programming. This enables the country team to be more purposeful and accountable with their health systems investments, setting annual targets and benchmarks that are similar to the annual treatment and service goals.

For example, a team may diagnose weaknesses in a lab system that will require the development of new labs, equipment purchases, new supply chains, and capacity building to operate the labs. The new Table 6 groups the activities logically and assigns benchmarks that monitor the scale-up and functioning of the new system while ensuring that the varied activities are coordinated and sequenced properly. In 2017, PEPFAR validated and updated the annual indicators to ensure they are well aligned with the desired programmatic outcome.

With the natural lag between science and implementation, Table 6 also ensures that new science and policies are adopted quickly and completely. Investments captured in Table 6 and the resulting approval process engenders the rapid identification and adoption of policies and programming to speed their implementation. Notably, the rapid adoption of the Test and Start policy that calls for HIV-positive individuals to start ART as soon as they are diagnosed was significantly accelerated through PEPFAR's focus on this critical policy to enhance viral suppression.

Table 6 also helps operationalize the Sustainability Index and Dashboard (SID). For PEPFAR, sustainability of the HIV response means that a country has the enabling environment, services,

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systems, and resources required to effectively and efficiently control the HIV/AIDS epidemic.

The Sustainability Index and Dashboard – A Road Map to Transfer Responsibility

The SID is a measurement tool, began in 2015, that provides a framework and periodic snapshot of the elements central to a sustained and controlled epidemic. The implementation of the SID allows PEPFAR to objectively track progress toward program sustainability goals.

The Index targets 15 elements organized under the following four overarching domains:

- Governance, leadership, and accountability
- National health system and service delivery
- Strategic investments, efficiency, and sustainable financing
- Strategic information

The specific indicators and milestones included in the SID measure key areas, including partner countries mobilizing domestic financial resources for their HIV/AIDS response and allocating those resources strategically and efficiently; collecting, analyzing, and using the right types of data for decision-making; and ensuring a secure, reliable, and adequate supply of and distribution system for drugs and other commodities needed to achieve sustainable epidemic control.

The SID data collection and validation process is conducted every two years, and FY 2016 was marked by a complete review and updating of the tool in anticipation of the biennial data collection that occurred in November 2017. This review process, which was conducted with headquarters and field input as well as with multilateral and country partners, confirmed the existing 15 elements as essential to a sustainable response. By confirming the basic elements, PEPFAR can publish a comparable time series of data, which charts progress toward sustainability. Country SIDs are publicly available and provide an important information base for governments, other donors, and civil society to reflect on where efforts and funding are most needed to remove barriers to reach and sustain epidemic control.

Sustainable Financing as a Key Priority

A stable financial resource base, mobilized both domestically and externally, is essential for sustainability and critical for long-term planning and decision-making. Because funding will always be limited, each dollar must be stretched to ensure that investments are strategic, effective, and cost-efficient.

For PEPFAR, financial sustainability is located at the intersection of epidemiology and economics. It is the ability to afford epidemic control and to secure a stable funding source for HIV treatment, prevention, and surveillance and the health system that supports the program after control is achieved. To ensure that the financing is available to achieve epidemic control, PEPFAR is doing the following:

- Focusing on the efficient use of existing resources to ensure that maximum performance is achieved with limited funding
- Standardizing and sharing budget and expenditure data with the Global Fund, partner governments, civil society, and other donors to develop a complete picture of existing HIV financing
- Engaging Ministries of Finance (MOFs) to ensure comprehensive HIV programs are developed and funded in

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national budgets with increasing shares domestically funded over time

- Working with partner governments and civil society to develop key systems, including secure procurement supply chains and financial management systems, to maintain services and epidemic control

PEPFAR took a number of steps to integrate domestic resource mobilization and sustainable financing into our programming. FY 2016 saw an important maturation of the USAID-led Sustainable Finance Initiative (SFI). SFI focuses on encouraging domestic resource mobilization in the delivery of HIV/AIDS services in PEPFAR-supported countries.

To ensure the sustainability of HIV programs and strengthen country ownership, SFI is investing to leverage domestic resources for HIV/AIDS and improve approaches to health financing. SFI helps governments improve efficiency to meet the coverage goals for 90-90-90/Test and Start through data-driven resource allocation for health and HIV/AIDS at the national and subnational levels. As plans are developed, four areas of assistance are considered: advocacy through information, improved public financial management, supporting insurance and risk pooling mechanisms, and strengthening the private sector.

Building a Data Platform

Transparent, accurate, and timely health, epidemiologic, performance, and financial/expenditure data are essential for making informed and impactful investments that drive long-term improvements in health care services and systems and lower costs. In addition, they allow for the type of active surveillance that enables a country to respond quickly to outbreaks and contain them before they become uncontrollable. Access to data builds ownership, enhances problem solving, promotes accountability, and allows for real-time decision-making that gets a country to epidemic control.

To achieve sustainable epidemic control, we are complementing our efforts to enhance Minister of Health (MOH) data capacity by forging innovative partnerships to support countries in building robust, wider data systems that engage all stakeholders and leveraging these systems to accelerate, focus, and sustain the response to HIV/AIDS.

The Data Collaboratives for Local Impact partnership between PEPFAR and the Millennium Challenge Corporation promotes data for decision-making at the community and national level in Tanzania and will expand to Côte d'Ivoire in 2018. More than 300 individuals, principally from the health sector, were trained. Data Collaboratives for Local Impact's Data for Local Impact Innovation Challenge recently awarded 10 grants for innovative data-driven approaches to support the DREAMS program. One Innovation Challenge winner is working with schools in Tanzania to build a predictive analysis tool to keep girls in school and lower their risk of contracting HIV.

Similarly, with PEPFAR's support, the Global Partnership for Sustainable Development Data (GPSDD) helps countries like Ghana, Kenya, and Tanzania engage stakeholders to develop data road maps to tackle development priorities. These efforts gained momentum at a June 2017 meeting hosted by Kenya and GPSDD, Data for Development in Africa, which featured innovative ideas on how to harness data to end AIDS. GPSDD's Leave No One Behind Data Collaborative promotes best practices on data disaggregation and citizen-generated data to ensure that the most vulnerable benefit from improvements in health, education, and equity that help reduce their risk of HIV/AIDS.

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On June 16, 2017, more than 500 people gathered to commemorate the Day of the African Child in Nairobi, Kenya, and to speak up publicly for the rights of children and adolescents living with HIV.

PEPFAR has partnered with Humanitarian OpenStreetMaps, USAID's YouthMappers, the Department of State's MapGive, and the Young African Leaders Initiative to engage youth in mapping more than 195,000 buildings and 44,000 miles of road in high-HIV-prevalence districts of Côte d'Ivoire, Kenya, Tanzania, and Uganda. These "mapathons" help PEPFAR and our partners make data-informed decisions and improve program impact, while inspiring young Africans to engage in AIDS-prevention efforts. Participants have mapped remote fishing settlements in Uganda's highest HIV-prevalence district, helping optimize HIV service delivery and outreach and facilitate linkage to care and treatment.

Table 6. Spending Breakdown

Long-term strategy countries invest in above-site activities mostly in the areas of strategic information systems (19 percent of above-site spending on average), laboratory systems (16 percent), surveys and surveillance (12 percent), and supply chain (12 percent). Overall, long-term strategy countries spend an average of 13 percent of their total planning levels on Table 6 activities.

- In **Kenya**, SFI supported training for 26 out of 47 County Health Teams in program-based budgeting, which shifts the budget focus from line item inputs to programmatic results, in addition to training in advocacy, to ensure budgetary allocations are made for HIV/AIDS services.
- In **Vietnam**, SFI is actively supporting transition of program financing and enrollment of HIV patients to Vietnam's Social Health Insurance system.
- Support for advocacy at the national level in **Tanzania** resulted in a first-time allocation of $4.6 million for ARVs in Tanzania's FY 2016/2017 budget.

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A couple in Kenya receiving HIV counseling in Kenya.

- In **Nigeria**, in the private sector, SFI supports market-based approaches for HIV service delivery to mobilize resources from clients in higher income brackets, providing $705,799 for HIV/AIDS programming in its first year.
- SFI supported the government of **Uganda** to adopt program-based budgeting and to increase to its annual government budget absorption rate from 79.9 percent in FY 2015/2016 to 96.41 percent in FY 2016/2017, thereby increasing the fiscal space for health and HIV/AIDS services.
- In the **Dominican Republic**, SFI is generating evidence and supporting advocacy efforts to cover more than $16 million of the cost of ARVs within the Dominican Republic's social health insurance scheme.

Engaging Partner Governments and Civil Society

For PEPFAR, sustainability means that a country has the laws, policies, services, systems, and resources required to effectively and efficiently control the HIV/AIDS epidemic. Sustainability demands a long-term effort to ensure that a country establishes and maintains requisite levels of fiscal ability, technical capability, political will, and citizen engagement. PEPFAR uses a sustainability framework that emphasizes a drive to control the epidemic to the point that the remaining disease burden can be financed by a partner country's resources and managed with its own technical capability.

In the past, PEPFAR has emphasized formal partnership frameworks to drive host-country

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stakeholders toward sustainability and self-sufficiency. Now, PEPFAR emphasizes that partnerships should be continuously evolving and embedded in all aspects of program development and execution using data analytics jointly. Embedding partnerships into daily operations encourages shared responsibility that engages all country stakeholders to develop a system that fits their needs and realities, with an eye toward full partner country responsibility in the future.

Engagement with civil society, including faith-based organizations (FBOs), is a strong driver of sustainability. PEPFAR encourages the full participation of civil society in every stage of our programming and planning, from advocacy to service delivery, as it is a key to the success and sustainability of PEPFAR and the global effort to address HIV. Civil society has been a leading force in the response to HIV/AIDS since the beginning of the epidemic, and this longstanding involvement has resulted in expertise and relationships with local communities that nonindigenous organizations often struggle to achieve. It is critical to ensure that community and civil society are meaningfully engaged and have a voice at the decision-making table.

Efforts to embed partnerships in normal program operations start with the development of a PEPFAR COP/ROP. More than ever before, partner governments and in-country Civil Society Organizations (CSOs) were involved in the development, planning, and approval of the 2017 COPs. Representatives from the MOH and CSOs, including FBOs, attended the 2017 COP in-person reviews.

Together, PEPFAR teams, PEPFAR headquarters representatives, multilateral colleagues, and MOH and CSO partners reviewed epidemiological and program data, allowing all stakeholders to analyze and understand the information that underpins PEPFAR program planning and decision-making. MOH presence allowed real-time, effective discussion concerning high-level policy decisions, including Test and Start, and ensured MOH buy-in on planned activities. CSO presence helped better integrate the concerns of civil society into COP planning, and in many instances, CSOs strongly advocated to move the PEPFAR teams forward to reach even more PLHIV with services during the COP 2017 implementation.

We developed the PEPFAR Oversight and Accountability Review Team (POART) process, which is a quarterly review of progress to identify weaknesses and areas that require midcourse adjustments. Results are reviewed in person with partner country stakeholders who are integral to identifying problems and bottlenecks that inhibit performance and to mitigating the problems with appropriate solutions and actions.

PEPFAR teams are building new relationships in partner countries. In 2016, the U.S. Department of the Treasury joined PEPFAR as an implementing agency with the important goal of engaging peer MOFs in the HIV/AIDS response. For example, in countries like Zambia and Uganda, Treasury advisors are facilitating MOF involvement in the development of country HIV plans and are supporting the establishment of HIV expenditure steering committees. Treasury is also working on a range of activities, including helping domestic resource mobilization efforts in PEPFAR-supported Technical Assistance/Technical Cooperation countries where the country scored low on the SID, and assisting in the execution of HIV budgets in long-term strategy countries.

As PEPFAR countries move toward more sustainable programs and transition to local ownership, many national governments will depend on civil society to an even greater extent to meet the health needs of their citizens. Meaningful engagement with PEPFAR builds the capacity of local CSOs to meet this challenge, better preparing them to play a leadership role now and in the future.

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Locally Based Partners

Sustainable epidemic control will not be possible without locally based partners. PEPFAR 3.0 reiterated that PEPFAR cannot achieve an end to HIV/AIDS alone; strong partnerships are critical. That is why PEPFAR seeks to foster locally led prevention, treatment, and care services by partnering with local organizations and institutions.

PEPFAR's Local Capacity Initiative (LCI):

The LCI began in 2012 provides funding to local nongovernmental organizations (NGOs) in 14 PEPFAR-supported countries to assist local CSOs in increasing the effectiveness and responsiveness of local health systems through expanding advocacy efforts at all levels. The LCI's goals include increased accountability and transparency of government, reduced legal and policy structural barriers, reduced stigma and discrimination for key populations, and an enabling environment for CSOs. Below are highlights and achievements stemming from LCI programming:

- The Cameroon Baptist Convention Health Board (LCI grantee) is working in local health districts to influence local ownership and financing of health activities, such as introducing a first-ever line item for health in local council budgets.
- The Central Asian Association of PLHIV (LCI grantee) and other stakeholders successfully convinced the Ministry of Health and Social Development in Kazakhstan to commit to utilizing the UNICEF ARV procurement system in 2016 to dramatically reduce the procurement cost of ARVs. All LCI countries undergo significant Policy and Advocacy Training with technical assistance providers.
- In India, LCI grantees used GIS hotspot mapping with CSO groups working with key populations. Through GIS, services can be mapped against hotspots to determine how to navigate high-risk clients into testing and care services.
- In Zimbabwe, CECHLA (LCI grantee) pioneered a Community Scorecard (CSC) System that tracks service provision and health worker attitudes at the clinic level. The CSC documented improvement in health worker attitudes and stigma against Key Populations (KPs). Several LCI countries are developing and implementing community scorecards to increase discussion/advocacy for stigma and discrimination. The CSC also encourages more transparency and accountability from service providers and empowers clients to take control of their own care, while also helping the clinics succeed. For example, the CSC process revealed that clinics were not open late enough for the entire community to use their services and, as a result, several clinics extended their hours to better meet the health care needs of their community.

Gender and Sexual Diversity (GSD) Trainings:

Building on the success of the in-country GSD trainings, the Health Policy Plus (HPP) project and PEPFAR developed a GSD blended learning package that includes online and in-person training components. The blended learning package requires all PEPFAR field and headquarters staff who were not trained in person to spend 90 minutes participating in the online interactive GSD curriculum, and all our staff must also join an in-person panel discussion with local gender and sexual minority representatives around HIV, human rights, and meaningful engagement of GSD in PEPFAR programming.

Thus far, more than 400 PEPFAR field and headquarters staff have taken the online training. HPP also continues to conduct yearly

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A group of young women in Uganda.

in-person trainings of CDC, USAID, and S/GAC headquarters staff. Many PEPFAR programs operate in countries where gender and sexual minorities face increasing violence, legal sanctions, and a disproportionate burden of HIV, and are further imperiled under hostile social and political conditions, making our efforts to scale up HIV programs increasingly difficult.

It is important that PEPFAR and partner staff understand the existing constructs around GSD and our impact on the HIV epidemic, especially among high-burden sexual and gender minorities. PEPFAR will continue to innovate in this area to ensure that our staff is knowledgeable and effective at serving our most vulnerable populations.

While PEPFAR moves forward in our drive to be more efficient and transparent, PEPFAR country teams will continue to expand their collaboration with local civil society, including activists, advocacy groups, and service delivery organizations, to ensure they are actively engaged in PEPFAR processes and in the country-level HIV/AIDS response. PEPFAR will also work to do the following:

- Expand PPPs to address critical issues and challenges faced by communities and key populations
- Ensure that programs such as the Key Populations Investment Fund, the Faith Initiative, and the Elton John LGBT Fund scale up quality HIV/AIDS prevention, care, and treatment programs
- Continue to work with stakeholders and partner governments to address social and structural factors (such as stigma, discrimination, violence, and human rights violations)
- Work more closely with partners such as community and CSOs, governments, UNAIDS, the Global Fund, and others to strengthen and coordinate efforts

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A group of mothers in Kenya.

Robert Carr Civil Society Networks Fund:

PEPFAR is a founding donor of the Robert Carr Civil Society Networks Fund (RCNF), along with the United Kingdom, the Netherlands, Norway, and the Gates Foundation, which aims to support and strengthen the capacity of global and regional civil society networks as strong partners in the delivery of HIV services and as champions of human rights. This investment helps bring often-marginalized populations out of the shadows and into prevention services and health care clinics. Through the RCNF, 18 global and/or regional networks have been funded in every geographic region of the world. The RCNF is especially important to supporting efforts aimed at key populations that include men who have sex with men (MSM), sex workers, transgender individuals, people who inject drugs, and all PLHIV – from young people who have lived with HIV since birth to older men and women who have survived more than a decade due to the success of HIV treatment.

By funding consortia of networks of CSOs, the RCNF is able to achieve "economies of scale" in civil society impact. RCNF provides grants to equip smaller entities to function as organizations, which are capable of producing budgets, running audits, and utilizing the latest technology. As a result, these groups have become a stable global platform for advocacy, able to engage in Global Fund and PEPFAR processes. In 2017, as a result of the investment of RCNF, one network in the MSM consortium, the Global Forum on MSM and HIV, outpaced the MSM consortia's own growth and became a principal recipient of Global Fund grants. This outcome was a result of core and infrastructure support provided by RCNF to build network capacity to have an impact.

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NGO Delegation to UNAIDS

In 2017, PEPFAR supported the UNAIDS Non-Governmental Organization (NGO) Delegation members from PEPFAR countries across all UNAIDS regions for a retreat held at the S/GAC offices in Washington, D.C. This provided NGO Delegation members the chance to explore key policy issues of UNAIDS and sharpen the program and advocacy expertise of the group to recommend new and pragmatic approaches that the UNAIDS Program Coordinating Board (PCB) could adopt in the short and long term. The impact of this training opportunity was evident through the inclusion of civil society recommendations into the final decision points of a very successful PCB at the end of the year. The group recommended a thematic day at the PCB that addressed discrimination in health care settings and saw the issues thoroughly discussed and the majority of decision points adopted. The opportunity presented by PEPFAR allowed the group a chance to work together, learn together, and develop strategies for the upcoming year. This best practice has now been embedded in the NGO delegation's annual work plan, with Germany and India offering to host the group in 2018.

PEPFAR and the Elton John AIDS Foundation (EJAF) continue a $10 million partnership, originally launched in November 2015, to provide grants to organizations working to meet the HIV-related needs of key populations, with an initial focus on sub-Saharan Africa. The EJAF and PEPFAR each invested $5 million to improve access to HIV services for key populations and to help create nonstigmatizing environments by working with community leaders, civil society, and service providers, and targeting projects that provide outreach and support to LGBT people in countries with a high HIV burden.

A Rapid Response Fund, managed by the International HIV/AIDS Alliance and the Global Forum on MSM & HIV, was created to support activities that respond to immediate and urgent threats to the key population community as well as longer term projects aimed at influencing legal, policy, or other developments deemed hostile to those communities. In addition, a Deeper Engagement Fund was created to support grants in Kenya, Uganda, and Mozambique that improve access to HIV prevention, care, and treatment information and services for key populations; decrease stigma and discrimination; and increase the capacity of related community-based CSOs.

Engaging Faith-Based, Locally Based, and Minority-Serving Partners

Ending AIDS by 2030 requires the global community to work together, including FBOs, locally based partners, and minority-serving institutions (MSI).

Faith-Based Organizations

From the earliest days of the epidemic, FBOs have been central to the HIV/AIDS response, both domestically and internationally. Compelled by their mission to care for the least fortunate among us, FBOs have been on the ground for decades, even centuries, providing care and treatment to communities outside the reach of public health systems and acting as a voice for the voiceless. In the global context, FBOs were among the first programs to respond to the particular needs of children infected with, affected by, and orphaned by HIV/AIDS.

Since the beginning of the epidemic, we have made great progress in reducing the impact of HIV/AIDS on families and communities around the world, and faith-based partners remain indispensable partners in the effort. In many PEPFAR countries, FBOs are the largest nongovernmental provider

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of HIV services. A PEPFAR-funded study in Kenya conducted across all 47 counties in the country found that only 22 percent of HIV treatment-related services were provided by health care service providers, excluding other services that may be provided such as HIV testing or PMTCT.[26] In Nairobi County, the highest-burden county in Kenya, faith-based health providers support 79 percent of children on treatment, and 40 percent of adults. These numbers demonstrate why PEPFAR has committed more than $1 billion in planned funding to more than 40 faith-based partners since 2012.

Revitalized and Increased Engagement with Faith-Based Organizations

In September 2017, the contribution of faith-based partners was recognized by the U.S. Department of State, and in the new PEPFAR Strategy, one of the strategic pillars is "renewed engagement with faith-based organizations... to accelerate and improve efforts toward epidemic control."

To that end, PEPFAR is expanding faith-based engagement in the following ways in 2018:

- Partnering to reach men and boys
- Partnering to reduce risk among young people
- Partnering to increase access to treatment for children and adolescents

Action Plan on Scaling Up Early Diagnosis and Treatment of Children and Adolescents

One of the most important facets of our commitment to work with faith-based partners is our shared focus on pediatric treatment and our ambitious goals to end AIDS in children, adolescents, and young women by 2020 as captured by the *Start Free Stay Free AIDS Free* Framework.

In November 2017, PEPFAR, along with the Vatican Dicastery for the Promotion of Integral Human Development; UNAIDS; Caritas

A nun greets a group of children in Lesotho.

[26] http://ihpemory.org/kenya-overview/kenya-high-incidence/

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International; the Ecumenical Advocacy Alliance, an ecumenical initiative of the World Council of Churches; WHO; and the Elizabeth Glaser Pediatric AIDS Foundation convened leaders of major pharmaceutical and medical technology companies, multilateral organizations, donors, governments, faith-based partners, and other key stakeholders for a High-Level Discussion on Scaling Up Early Diagnosis and Treatment of Children and Adolescents. Participants gathered to discuss ways to expedite the research, development, approval, introduction, and uptake of optimal drugs and formulations for infants, children, and adolescents.

Building on meetings held in 2016 and 2017, the High-Level Dialogue provided an opportunity for stakeholders to put forward a set of concrete actions to better support the research, development, and introduction spectrum. Following the meeting, a new Action Plan for Scaling Up Early Diagnosis and Treatment of Children and Adolescents was released on World AIDS Day 2017, which was endorsed by stakeholders from all sectors and built on commitments within the Global Accelerator for Pediatric Formulations (GAP-f) and the *Start Free Stay Free AIDS Free* framework.[27] In this plan, the participants agreed to focus, accelerate, and collaborate on the development, registration, introduction, and rollout of the most optimal pediatric formulations and diagnostics.

PEPFAR is fully supportive of the new Action Plan and will be taking positive steps in 2018, including additional assistance and funding of research to inform development, approval, and use of pediatric formulations, and supporting actions required for quick introduction and scale-up of new, optimal pediatric formulations.

A key reason for our global success is the role of the U.S. Food and Drug Administration and its willingness to create a workable pathway for earlier access to safe and effective drugs. Recently, they noted a new, innovative approach that brings a parallel drug development process for the most vulnerable populations we serve: infants and children.

At the conclusion of the Vatican Consultation, Pope Francis released the following statement: "*Health care strategies aimed at pursuing justice and the common good must be economically and ethically sustainable. Indeed, while they must safeguard the sustainability both of research and of health care systems, at the same time they ought to make available essential drugs in adequate quantities, in usable forms of guaranteed quality, along with correct information, and at costs that are affordable by individuals and communities.*" This is in line with PEPFAR's goals and with Ambassador Birx's leadership in the effort to end pediatric HIV.

PEPFAR-UNAIDS Faith Initiative

We know that to help our faith-based partners contribute to a truly sustainable HIV/AIDS response, we must equip them to do more than service provision funding. PEPFAR currently contributes to local capacity building and technical assistance, particularly in the area of data collection and analysis.

In July 2015, The Lancet published a seminal series on faith-based health care that called for more robust data on the contributions of faith-based health providers. PEPFAR is committed to data for impact, and, in response to The Lancet series, PEPFAR, in partnership with UNAIDS, announced the PEPFAR-UNAIDS Faith Initiative. This is a $4 million initiative to fast-track the faith-based response to achieve the UNAIDS 90-90-90 goals by strengthening the capacity of faith-based leaders and organizations to advocate for and deliver a sustainable HIV/AIDS response.

FBOs are powerful voices in making national policies, calling for justice, protecting the

[27] http://www.pedaids.org/page/-/Rome_Action_Plan_2017.pdf

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rights of the marginalized, addressing stigma and discrimination, and mobilizing people to employ testing, treatment, and prevention services. This PEPFAR-UNAIDS Faith Initiative aims to recognize and harness the strengths of the faith community in the HIV/AIDS response. The focus areas are as follows:

- **Collect, analyze, and disseminate data** on health care services provided by faith-inspired organizations
- **Address stigma and discrimination** in communities and health care settings
- **Create demand for service uptake and retention in care** by building the capacity for joint action between communities of PLHIV and faith to increase demand for HIV services and retain people in care
- **Strengthen HIV- and AIDS-related service provision** by strengthening networks of faith-based health service providers – Christian, Islamic, and others – to reach the most marginalized and at-risk populations with comprehensive, equitable HIV testing, prevention, and treatment services through strengthened national partnerships, improved data collection, and careful monitoring and evaluation
- **Strengthen FBO leadership and advocacy** for the fast-track approach and a sustained AIDS response to end the global AIDS epidemic by 2030

In 2017, PEPFAR's faith-based partners concluded their work on Phase I of the initiative, focusing on Kenya and Zambia. Phase II began in Democratic Republic of the Congo, Nigeria, Tanzania, and Zimbabwe. On the ground, faith-based partners are engaged in the following:

- Research on FBO service delivery
- Research on stigma and discrimination in faith-based health care settings
- Demand creation and addressing stigma and discrimination in communities
- Building stronger faith-based health systems

Simultaneously, PEPFAR's faith-based implementing partners were active in the United States and abroad, strengthening FBO leadership and advocacy for a sustained, fast-track approach to end the AIDS epidemic by 2030.

In June 2017, Caritas International, with PEPFAR support, convened a regional multistakeholder consultation in Abuja, Nigeria, focused on the early diagnosis of and treatment for HIV-positive children. Outputs of this meeting included plans to strengthen collaborative national partnership and national action plans, and highlight opportunities for cross-country exchange of lessons learned and replicable models. The discussions at the Nigeria Consultation informed Phase II programming in partner countries.

Adjacent to the September 2017 United Nations General Assembly Meeting, with PEPFAR support, Ecumenical Advocacy Alliance – World Council of Churches hosted its annual high-level interfaith prayer breakfast, Fostering Partnerships for Fast-Tracking Access to Testing and Treatment for Infants, Children, and Adolescents, which brought together leaders from all major church traditions, government, private sector, and other NGOs, and served as an opportunity for all stakeholders to recommit to our shared goals.

Our faith-based partners are key stakeholders in the HIV/AIDS response. Their role needs to be better recognized, understood, and supported, not only by PEPFAR, but also by all global funding institutions. In many countries hardest hit by the epidemic, religion plays an important role, both spiritually and as a source of information

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A mother visits a health clinic with her baby in Tanzania.

and inspiration. It is essential for PEPFAR to continue our engagement with FBOs as the global community begins to tackle the most intractable barriers to access to treatment, including stigma and discrimination. The commitment of FBOs and faith leaders to service, nonjudgmental support, and universal access serve as an example to the U.S. government as we engage in care and treatment around the world.

U.S. Minority-Serving Institutions (MSI)

In 2017, PEPFAR supported the Historically Black Colleges and Universities (HBCUs) of Charles R. Drew University of Medicine and Science, Meharry Medical College, Morehouse School of Medicine, and Howard University College of Medicine and their African-based teaching and training institutions in the creation of an HBCU Global Health Consortium. The Consortium works with health care worker training centers and clinics to transform clinical HIV practice to provide high-quality, comprehensive, and professional care and treatment to PLHIV.

The project is designed to train HCW within high-burden HIV settings on how to reduce stigma and discrimination and on how the social determinants of health impact health outcomes along the HIV care continuum. After the project launch in February 2017, the Consortium conducted a clinical needs assessment site visit in Zambia, the first country selected, this summer. In December 2017, implementation of site-level clinical practice transformation will start with a follow-up visit to Zambia.

Additionally, the PEPFAR Scientific Advisory Board includes experts affiliated with HBCUs, such as Celia Maxwell of Howard University and Lejeune Lockett of Charles Drew University of Medicine and Science, and the faith community, such as Reverend Edwin Sanders of the Metropolitan Interdenominational Church of Nashville and Nyambura Njoroge of the World Council of Churches.

Engaging International and Nongovernment Partners & Coordination with Multilaterals

Global Fund

The Global Fund is a multilateral financing mechanism that relies on public and private contributions on a three-year replenishment cycle. The Global Fund is a partnership between donor countries, the private sector, private foundations, implementing governments, civil society, international organizations, and affected communities. This partnership governs, oversees, and implements the Global Fund strategic vision of ending HIV/AIDS,

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TB, and malaria while building resilient and sustainable systems for health.

The Global Fund was established in 2002 as an independent nonprofit foundation. Since the Global Fund's inception, the United States is its largest single donor and its largest technical resource for supporting program delivery at the country level. The United States views the Global Fund as additive to its bilateral programs in HIV, TB, and malaria, and an additional mechanism to achieve the goal of HIV epidemic control and reduction of TB and malaria morbidity and mortality. As with its bilateral programs, the United States prioritizes performance against funding – in this case, the Global Fund delivering the greatest possible impact for those most in need with its investments.

The United States is a leader in making financial and policy contributions to the Global Fund. Since 2002, the United States has contributed $12.92 billion (as of December 30, 2017) to the Global Fund, abiding by limits on U.S. contributions to no more than 33 percent of the total amount of funding contributions as specified in applicable U.S. law. For the current fifth replenishment period (2017–19), the maximum pledge from the United States will be $3.84 billion over the three-year period. Additionally, the United States is an active presence in the Global Fund's governance structure, including a seat on the Global Fund Board of Directors, and each of the three Board subcommittees: Strategy, Audit and Finance, and Ethics and Governance.

As a financing institution, the Global Fund operational model does not include an in-country presence. PEPFAR's bilateral programming serves as a strong partner to the Global Fund, providing in-country intelligence and advice. The Global Fund Secretariat sees PEPFAR and the President's Malaria Initiative as essential contributors to shaping the content of in-country grants. The same approach with the Global Fund Secretariat is fostered in USAID TB programming. The U.S. investment in the Global Fund is additive to the PEPFAR program, bolstering our bilateral program results, expanding the geographic reach of the U.S. global health response and investment, promoting sustainable country-owned responses to the three diseases, and attracting continued investments from other donors to the Global Fund.

Since the beginning of our global response to the three diseases, it is evident that no one country or one institution can accomplish the mission of controlling the HIV/AIDS pandemic alone. Only through the complementary goals set by the leading institutions in the global health space, including PEPFAR, UNAIDS, and the Global Fund, can this be achieved. In July 2017, the Global Fund reported the global HIV, TB, and malaria results, which were achieved by countries through the collective effort of the Global Fund, U.S. bilateral programs, national leadership, and other catalytic donor support. Significant reductions in deaths from HIV, TB, and malaria were reported: 22 million lives saved since 2002 and 11 million on ART. In line with PEPFAR priorities, the Global Fund is moving to evaluate its performance against funding in 25 countries that receive more than 80 percent of the available funding.

In the past year, the Global Fund began to enact its new strategy, *Strategy 2017-2022: Investing to End Epidemics*. The Global Fund Strategy has four key strategic objectives:

- Maximize impact against HIV, TB, and malaria
- Build resilient and sustainable systems for health
- Promote and protect human rights and gender equality
- Mobilize increased resources

Responding to the call to increase transparency on the impact of Global Fund investments and

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A group of young girls in Namibia create an AIDS ribbon out of soda cans to bring awareness to HIV/AIDS.

linking program results to funds expended, the Global Fund made significant changes to its reporting requirements for grant recipients and the level of detail of results reporting to the Board and Board committees.

Commencing with the allocation period 2017–2019 and program results reporting in 2018, country grant recipients must report on a standardized set of program indicators that shifted from output-based metrics (e.g., persons trained or HIV test kits procured) to outcome metrics that are connected with program impact (e.g., number of persons tested for HIV and percent HIV-positive). The Global Fund Secretariat will provide a report of disaggregated and aggregate-level results reporting across countries and by disease based on data collected as part of the grant results performance frameworks. While the Global Fund reports on national country-level results, it will move to a more transparent contribution reporting through Country Results Profile reporting.

The Country Results Profiles will be done for 25 countries, representing 80 percent of the Global Fund investments. The Results Profiles will show the financial level of Global Fund investment against the national results reported for a specific program area, thus clarifying the level of Global Fund investment in a given program area. For example, if the Global Fund invests 60 percent of a country allocation in ARVs and finances 90 percent of ARV procurement for that country, it will be held to account for positive or negative numbers of new persons enrolled in treatment and persons retained on treatment.

Lastly, the Global Fund will report on a new set of Key Performance Indicators for the institutions that are aligned with the major objectives of the Fund Strategy (2017–2022). This will not only provide the United States with improved visibility on the full scale of the Global Fund investments, but also allow the U.S. government to ensure that investments are being made efficiently and effectively.

Additionally, there were leadership changes at the Global Fund in 2017. In May, former Global AIDS Coordinator Mark Dybul ended his tenure as Global Fund executive director. Following a robust and transparent search process, the Global Fund Board of Directors selected a new executive director in November 2017. The board appointed Peter Sands, a former CEO of Standard Chartered Bank and a Global Health fellow at the Harvard Kennedy School.

The Global Fund Board leadership also changed in May 2017, with Ambassador John Simon selected as the vice-chair of the board; he will hold that post until May 2019. The U.S. remains committed to the mission of the Global Fund and working with its board to end the three pandemics.

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PEPFAR's ambitious goals to end HIV as a public health threat will be assisted by the investments made by the Global Fund. Our shared challenges – issues faced by women and girls, the need for sustainable and robust health systems that will serve the three diseases as well as contributing to efforts to address global health security and noncommunicable disease threats, and human rights – can be converted into successes by continuing this effective partnership. The Global Fund as an attractive platform for investors in the health sector of developing economies is a continued goal of PEPFAR.

UNAIDS

UNAIDS coordinates global efforts to end the HIV/AIDS epidemic by bringing together, and guiding the efforts of, the UN system, governments, civil society, and the private sector. It coordinates 11 UN bodies to end HIV/AIDS as a public health threat globally by 2030. UNAIDS is a global norm-setting organization for the HIV/AIDS response that convenes partners and stakeholders for the purposes of collecting and reporting the best strategic public health information, and ensuring that the UN system's response to HIV/AIDS is coherent and unified.

The United States works with UNAIDS to make tangible progress on addressing HIV/AIDS, a long-standing U.S. policy priority. Using our position as a member state on the UNAIDS Board, the United States is able to leverage the resources and commitments of other member states' donor groups to support HIV/AIDS efforts and promote evidence-based policies and strategies to facilitate a more effective global response to the pandemic.

PEPFAR prioritizes working with and through others to build political will, particularly for much-needed policies that will help control the pandemic and sustain our joint impact on treatment and prevention, establish international norms, and ensure a broad-based multisector response to enhance and support service delivery. Through PEPFAR, the U.S. government supports and advances the UNAIDS 90-90-90 goals: 90 percent of people with HIV diagnosed, 90 percent of those diagnosed on treatment, and 90 percent of those on treatment virally suppressed by 2020. UNAIDS joined PEPFAR in 2017 to implement the new PEPFAR strategy to demonstrate a road map to epidemic control in 13 countries.

UNAIDS's policy framework and the political commitment to eradicate HIV/AIDS complement the goals of PEPFAR and the Global Fund. The United States relies on UNAIDS for data and a strategic framework to ensure that programs implemented through PEPFAR are effective and sustainable. As a multilateral organization, UNAIDS is also viewed as a neutral policy arbiter. In addition, UNAIDS's coordination and partnership with national governments enables alignment with country needs and priorities to be successful for the United States.

In FY 2017, Global Fund Technical Assistance resources supported UNAIDS Technical Support Facilities, technical assistance programs administered by UNAIDS to provide vital support to scale up HIV programs. Funding impacted 62 countries in Africa and Asia.

Achievements were noted in the operational, management, and financial systems to implement Global Fund-supported programs for 30 Global Fund Principal Recipients (PRs) and sub-Recipients (SRs); strengthened monitoring and evaluation systems for 10 Global Fund PRs and SRs; improved procurement and supply management systems for eight Global Fund PRs; supported four countries to revise policies that were barriers to implementation; supported 23 countries to develop or revise national strategic frameworks to fully align with the Fast-Track targets; conducted modeling analysis of different funding options for power for revenue generation undertaken in six countries and one region;

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CREDIT: USAID

A baby receives medical care at a clinic in Lesotho.

developed subnational strategic frameworks in 11 countries; developed fast-track action plans in 21 cities across six countries; delivered technical support for innovative service delivery models in eight countries; and conducted transition and financial sustainability assessment and planning conducted in six countries and two regions.

Targeted Public-Private Partnerships for Impact

Partnerships with the private sector play a critical role in advancing progress toward ending the HIV/AIDS epidemic. PEPFAR strategically focused our PPPs to increase programmatic impact and efficiency. PEPFAR's PPP strategy includes finding opportunities where the private sector can complement PEPFAR goals and priorities by leveraging private sector brands, distribution networks, innovation, technology, and market-driven approaches to help achieve epidemic control. Much like the private sector, PEPFAR is focused on accountability and scale. PEPFAR often looks to business models of private sector companies for ideas on how to run the program most effectively and efficiently. PPPs enable PEPFAR to not only share risks, resources, and rewards but also find greater efficiencies in program delivery.

In 2017, PEPFAR developed, implemented, and sustained 16 global PPPs. In addition to achieving greater efficiencies within the program, these partnerships demonstrate PEPFAR's continued commitment to achieving epidemic control among children, adolescent girls and young women, boys and men, and key populations. Some of these partnerships are highlighted below.

Achieving Greater Efficiencies in PEPFAR Programs

MasterCard

Innovative technology and service delivery models can help achieve greater efficiency in

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PEPFAR's efforts. At the 2017 Concordia Annual Summit, PEPFAR launched a new and pioneering partnership with MasterCard aimed at accelerating progress toward HIV/AIDS epidemic control through the use of digital technologies and data analytics. Leveraging these tools, the partnership intends to improve access to and outcomes of HIV/AIDS prevention and treatment services in resource-limited settings throughout Africa.

Project Last Mile

In an effort to increase efficiencies within the supply chain, PEPFAR partnered on Project Last Mile with the Coca-Cola Company, USAID, the Global Fund, and the Gates Foundation. The partnership aims to build supply chain and distribution capability in African Ministries of Health. Through the partnership, Coca-Cola shares its logistics, supply chain, distribution, and marketing expertise to help African governments maximize their own capacity to deliver critical medicines and medical supplies the "last mile" to remote African communities.

To date, Project Last Mile has reached regions within seven countries: Ethiopia, Ghana, Mozambique, Nigeria, South Africa, Tanzania, and Zambia. The partnership has helped establish systems to track stock products, create human resource systems that allow governments to evaluate employees' objectives and performance, and benchmark private sector spending on third-party services to ensure optimal use of public funds.

Labs for Life

PEPFAR has made remarkable contributions to ensure partner countries have sustainable laboratory systems to accurately detect, confirm, treat, and monitor disease in developing countries. Efficient diagnostic measures require strong laboratory systems, and PEPFAR's efforts have

CREDIT: USAID

Through the President's Emergency Plan for AIDS Relief (PEPFAR), the American people provide 65 percent of the monthly ARV therapy needed for 600,000 Kenyans living with HIV to enable them to live productive lives.

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contributed to not only control of HIV, but also to the larger global health security agenda. The next phase of the Labs for Life partnership with Becton Dickinson was renewed in 2017 and will continue to build capacity for laboratory systems to improve HIV and TB diagnosis, treatment, and monitoring in countries that it supported in Phase II. Additionally, it will leverage support to key countries where weak laboratory systems are an obstacle to meeting the goals of epidemic control.

Pink Ribbon Red Ribbon

Pink Ribbon Red Ribbon (PRRR) was formed to address cervical and breast cancer in low- and middle-income countries in sub-Saharan Africa, especially for high-risk HIV-positive women. PRRR leverages the PEPFAR platform to provide care for women infected with HIV and other associated conditions, particularly cervical cancer. The focus on high-risk HIV-positive women is important to PEPFAR given that women with HIV infection are four to five times more likely to develop cervical cancer, compared with HIV-negative women. As of the second quarter of 2017, 99,980 women had been identified as HIV-positive through a basic screening for cervical cancer. The integration of HIV and cervical cancer services has been an effective and efficient method of responding to both diseases.

Finding Men to Break the Cycle of Infection

More than half of men under age 35 do not know their HIV status and are not on treatment, fueling the epidemic, especially among women ages 15–24. PEPFAR is working with several private sector partners to find innovative solutions to find and reach at-risk men under age 35 to increase their rates of HIV testing and overall interaction with the health care system. In late 2017, PEPFAR brought these private sector partners together to source ideas on core competencies and subject matter expertise in consumer marketing, data analytics, and product innovation that could be applied to addressing this challenge. A new PPP will leverage shared public health and business value in investing in innovative initiatives to engage men in quality care services.

Healthy Heart Africa, with AstraZeneca

There is an urgent need for innovative approaches to better identify and serve harder-to-reach populations, including men, who too often only seek care when they are very ill. Through a partnership with AstraZeneca, PEPFAR is enhancing our ability to deliver earlier and more effective HIV/AIDS testing and treatment for working-age men ages 25–50 in Homa Bay and Kisumu, Kenya. PEPFAR seeks to demonstrate that an integrated HIV-hypertension service delivery model results in efficiency and greater synergy in the identification, linkage to care, and treatment of men with HIV/AIDS.

Elizabeth Taylor AIDS Foundation (ETAF)

In Malawi, as in many other countries, data show that men often access HIV testing at far lower rates than women. As a result, men living with HIV often enter care with more advanced disease, are less likely to receive lifesaving antiretroviral therapy, miss opportunities to prevent ongoing transmission, and have higher AIDS-related mortality than their female counterparts. Prevailing cultural norms around masculinity in Malawi also can act as an additional barrier to men accessing HIV services.

PEPFAR partnered with the Elizabeth Taylor AIDS Foundation (ETAF) to accelerate progress toward controlling the AIDS epidemic in Mulanje, Malawi, among men ages 25–40. PEPFAR and ETAF combine resources and expertise to refine and innovate HIV testing and treatment strategies for identifying and serving men. For example, the partners used risk assessment tools to screen for high-risk behavior, which led to a more targeted testing strategy, resulting in higher HIV testing yields through more efficient testing modalities.

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CREDIT: Srgey Baranuk, PSI Central Asia

A patient receives a rapid HIV test in Kazakhstan.

Delivering on Our Commitment to Children

Accelerating Children's HIV/AIDS Treatment (ACT)

In 2013, only 24 percent of children living with HIV were receiving ART. In response, PEPFAR and the Children's Investment Fund Foundation launched the $200 million ACT Initiative, which represented an unprecedented investment of resources and technical expertise to achieve greater efficiencies. At the end of the two-year partnership, ACT was supporting 561,610 children (19 years or under) living with HIV with access to high-quality treatment in the nine ACT high-priority countries in sub-Saharan Africa. Additionally, pediatric ART coverage increased by 10 percent or more from 2014–2015 in most of the ACT countries – Kenya, Lesotho, Malawi, Tanzania, Zambia, and Zimbabwe – with Zimbabwe reaching 80 percent pediatric ART coverage.

Ensuring Access to Essential Prevention and Treatment Services for Key Populations

Elton John AIDS Foundation – LGBT Fund

In many countries, despite the significant progress made in addressing HIV/AIDS in the general population, LGBT populations often experience higher rates of HIV infection and more limited access to HIV/AIDS services than the general population. In response, PEPFAR launched the LGBT Fund in partnership with the EJAF to expand access to nondiscriminatory HIV-related services for key populations that lack access to adequate HIV services. As part of the overall partnership, a Rapid Response Fund was established to provide support for LGBT individuals and organizations requiring an emergency response and supported 115 grants across 20 countries. Additionally, a Deep Engagement Fund worth $6 million was formed and is providing larger grants to several organizations in Kenya, Mozambique, and Uganda.

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Strengthening Health Training and Data Systems

Human Resources for Health (HRH)

PEPFAR supports partner countries to increase their human resources for health (HRH) in order to deliver HIV services where the epidemic is most acute. Our HRH investments ensure that health workers with the right skills are in the right places to scale up HIV services at the right time to achieve UNAIDS' 90-90-90 goal. PEPFAR 3.0's HRH Strategy focuses investments on supporting the delivery of HIV services to priority populations in PEPFAR-supported sites and geographic areas by doing the following:

- Assessing HRH capacity
- Improving HRH information systems and data use
- Supporting HRH supply and retention
- Improving service quality
- Ensuring sustainable financing for health workers providing HIV services

To support this ambitious strategy, PEPFAR committed $110 million at the above-service delivery level at the end of FY 2016 to strengthen the capacity of health workers and country HRH systems to address HIV/AIDS across Africa. Additionally, specific funding increased the supply of skilled clinicians available to provide HIV services and supported the National Institutes of Health Fogarty International Center to enhance the capacity of key training institutions in Africa.

HRH investments are being leveraged to address the drivers of HIV and other health epidemics through health professions education programs implemented by the Health Resources and Services Administration (HRSA). Funding further supports Information Systems by the National Institutes of Health Division of AIDS to continue to ensure PEPFAR invests in evidence-based interventions.

In addition to these investments, USAID and CDC have continued to strengthen HRH to strategically expand the quantity and quality of health workforces to reach the UNAIDS 90-90-90 goals. In addition to above-service delivery investments, PEPFAR has provided financial and nonfinancial support to facility- and community-level health workers through salaries, in-service training, and benefits.

- PEPFAR's HRH Strategy has enhanced HRH programming by increasing the availability, quality, and retention of HCW, resulting in the improved delivery of HIV/AIDS services. In FY 2017, PEPFAR supported preservice training of an additional 29,750 workers to strengthen country capacity for the delivery of HIV and other health services.
- In Malawi, coordinated support from USAID and CDC has resulted in the recruitment and deployment of 378 health workers to support the government of Malawi, relieve work pressures in the facilities constraining HIV service delivery, and provide job opportunities for health workers who were unemployed due to a multiyear government freeze on recruitment. The HCW recruited included nurse midwife technicians, clinical technicians, laboratory assistants, medical assistants, and pharmacy assistants. Many of the health workers are PEPFAR scholarship beneficiaries and were previously unemployed despite the critical need for trained health workers in the country. Working in partnership with key government authorities, agreements are in place to transition health workers to government payroll after a period of PEPFAR support. These efforts serve as a best practice for other countries.

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- In Uganda, the HRH2030 program undertook an analysis to build a stronger investment case in HRH for increased domestic resource contribution and to guide the more strategic and efficient use of resources for HRH to support HIV/AIDS services. A methodology was developed to analyze HIV-related HRH costs, fiscal space, and the political economy of HRH financial decision-making. The analysis showed that a rollout of differentiated care models in Uganda could result in an estimated $1.7 million in HRH cost savings by 2020. Using the methodology developed, HIV investment cases can be used across PEPFAR country HRH stakeholders to advocate for increased domestic contributions for HRH needs to support HIV services, particularly in countries where PEPFAR provides significant HRH remuneration support.

- To assess HRH constraints and needs for continued implementation of Test and Start guidelines and differentiated service delivery models, PEPFAR further developed and applied rapid HRH facility assessments in Cameroon, Ethiopia, and South Africa.

- Unresolved facility-level health workforce bottlenecks can seriously hinder HIV services and efforts to achieve epidemic control. In response, the HRH2030 program developed a complementary resource to provide service delivery partners and facility managers with easily accessible and quickly applied tools to scale analysis and address common HRH-related performance and productivity gaps inhibiting HIV service delivery impact. In FY 2017, trainings were delivered in Nigeria and Tanzania.

- In Mozambique, the MOH successfully used the Workforce Allocation Optimization Tool for the last three rounds of allocation (December 2015, July 2016, and December 2016) for all midlevel cadres and more than 2,500 graduates. As a result, the MOH improved HRH retention and had far fewer reallocation requests, thereby saving significant HRH transfer costs.

- Health worker shortages pose a challenge to the scale-up of HIV care and treatment in Uganda. Training midlevel practitioners (MLP) in the provision of HIV and TB treatment can expand existing health workforce capacity and increase access to HIV services. In Uganda, the MENTORS project conducted a cluster-randomized trial of one-on-one, on-site clinical mentorship on HIV and TB care among 40 MLP in 10 health facilities in rural Uganda. MLP mean scores for knowledge increased by 14.5 percent and competence by 27 percent in the intervention arm relative to the change in the control arm. One-on-one, on-site mentorship improved HIV and TB knowledge and competence, has a downstream effect on facility performance, and is a simple approach to training MLP for task-sharing.

- Through funding to the African Health Professions Regional Collaborative, PEPFAR supported national nursing leaders from 14 countries in sub-Saharan Africa to improve the quality of HIV services in health facilities. Through CDC, PEPFAR provided small grants and facilitated peer-to-peer learning among nurse leaders from MOH, health professional regulatory councils, professional associations, and training institutions in order to advance site-level and national-level quality improvement for nurses who comprise the bulk of the health workforce in these countries.

Evaluation Standards of Practice

Background

In January 2014, we issued the PEPFAR Evaluation Standards of Practice (ESOP),

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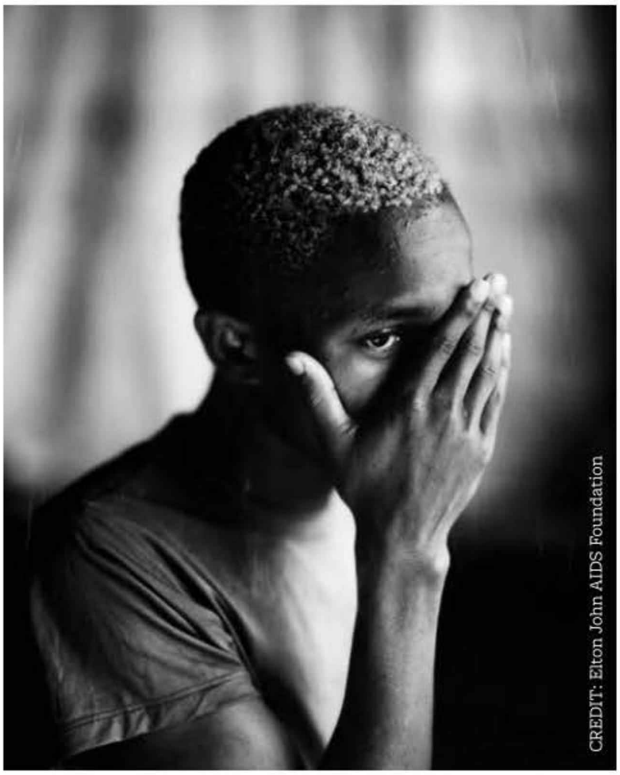

PEPFAR has specific initiatives addressing the dynamics driving stigma, discrimination, and violence as a part of our broader efforts to expand key populations' access to and retention in quality HIV/AIDS prevention and treatment services.

and in September 2015, released version 2.0.[28] The second document retained the original 11 standards and also provided operational guidance regarding requirements for annual planning (COPs/ROPs) and reporting processes (Annual Program Results). ESOP 3.0, which includes the same standards and some additional types of activities that must be reported on, was released in December 2017.

PEPFAR defines evaluation as the "systematic collection and analysis of information about the characteristics, outcomes, and impact of programs and projects."[29] All PEPFAR evaluations, regardless of the implementing agency, partner, or type of evaluation, must adhere to these standards.

In FY 2017, country and regional programs were asked not only to report on the four types of evaluations (process, outcome, impact, and economic) as in previous years, but also on IS and operations research (OR) activities. Expanded definitions, timeframes, indicators, and examples of questions can be found in ESOP 3.0. An Adherence Checklist, the tool that assesses completed evaluations against the standards, must be submitted for all evaluations and IS/OR activities.

The Standards of Practice

The 11 evaluation standards were developed and agreed upon by representatives from PEPFAR implementing agencies. The 11 standards are listed below, and full descriptions can be found in all of the ESOP documents cited above.

1. Engage stakeholders
2. Clearly state evaluation questions, purpose, and objectives
3. Use appropriate evaluation design, methods, and analytical techniques
4. Address ethical considerations and assurances
5. Identify resources and articulate budget
6. Construct data collection and management plans
7. Ensure appropriate evaluator qualifications and independence
8. Monitor the planning and implementation of evaluations
9. Produce quality evaluation reports
10. Disseminate results
11. Use findings for program improvement

[28] PEPFAR. (2015). Evaluation Standards of Practice – Version 2.0. Retrieved from http://www.pepfar.gov/documents/organization/247074.pdf
[29] Patton, M. Q. (1997). Utilization Focused Evaluation: The New Century Text. (pp. 23). Sage Publications.

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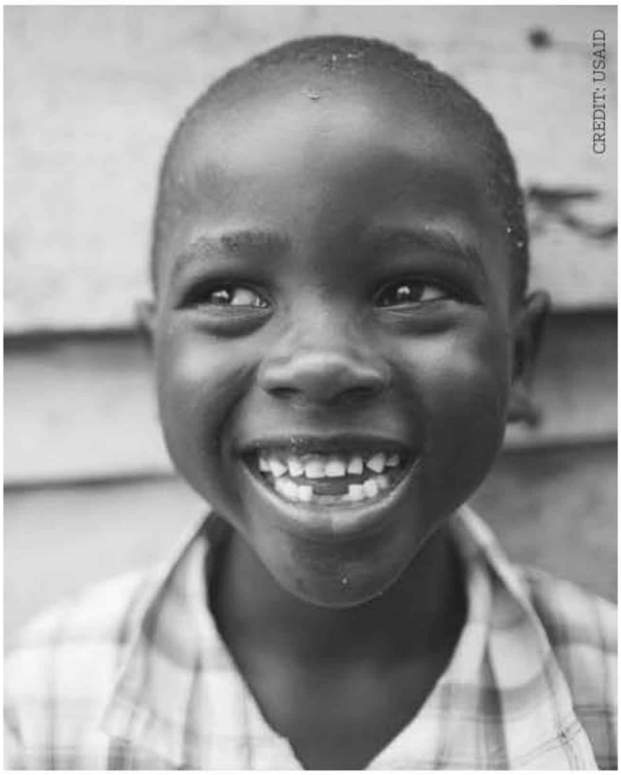

A young boy in Uganda.

Methods

This report includes a presentation of overall findings from evaluation, IS, and OR submissions in PEPFAR's Data for Accountability, Transparency, and Impact Monitoring (DATIM) system for FY 2017. FY 2017 is the fourth year for submission of evaluation results. Some evaluations started in years prior to the release of the ESOP in 2014, and as such, some flexibility was allowed for evaluations that began before the release of the standards. Agencies reviewed, verified, and assessed the evaluation, IS, and OR data submitted for PEPFAR's 2017 APR process, each using an agency-specific process. Results from the agencies were aggregated for this report.

Determining adherence to the standards is dependent on a review of a final evaluation report using the Adherence Checklist to answer a series of review criteria associated with each standard. Responses to these criteria include yes, partial, and no. For composite standards based on several questions, if all answers were yes, the final score was yes; if all were no, the final score was no; and any other combination of answers was given a partial score. The data presented were verified to assess completeness and confirmed to be completed during the reporting period and meeting the PEPFAR ESOP definitions of evaluation, IS, or OR activities.

Findings

Overall, a total of 314 evaluation, IS, and OR submissions were reported in FY 2017. In FY 2016, there were a total of 94 completed evaluations.[30] In FY 2017, the number increased to 109 completed evaluations. USAID had a total of 142 entries, of which 50 were completed evaluation, IS, and OR activities, and the remainder are in planning and implementation stages. The 50 completed evaluations represent activities in 19 PEPFAR countries, with Vietnam completing the highest number of activities (14). Of the 50 completed evaluations, seven are process evaluations, 15 outcome evaluations, three impact evaluations, and one economic evaluation. The new additions of IS and OR in FY 2017 reporting included 15 IS activities and five OR activities. Four activities were classified as "other."

CDC had a total of 172 entries, of which 59 were completed evaluation, IS, and OR activities, and the remainder are in the planning and implementation stages. The 59 completed evaluations represent activities in 18 PEPFAR countries, with South Africa completing the highest number of activities (nine).

Of the 59 completed evaluations, 27 are process evaluations, 25 outcome evaluations, two impact evaluations, and one economic evaluation. The new additions of IS and OR in FY 2017 reporting included two IS activities and one OR activity.

[30] Due to the extensive data cleaning and verification process that occurs after the annual reporting deadline (November 15, 2017), the actual number of completed evaluations and reporting of the standards based on submitted Adherence Checklists presented in this report may change.

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One activity was classified as "other." The U.S. Department of Defense and HRSA reported a number of evaluations at different stages, including one completed evaluation for HRSA.[31]

PEPFAR legislation requires reporting on the number of completed evaluations that are publicly accessible within the fiscal year. Note that this is separate from Standards of Practice 10, which relates to public dissemination within 90 days of completion. Overall, 76 percent of the FY 2017 evaluation, IS, and OR activities have been publicly disseminated, which is a slight decrease from 89 percent in FY 2016. Agencies are working to further increase the timely dissemination of results on publicly available websites. The dissemination of findings is critical to ensuring that results are used in a timely manner to make critical decisions. PEPFAR and our implementing agencies will continue making efforts to ensure stakeholders are aware of the importance of the requirement to disseminate results, and we expect to see improvements on this in subsequent years.

Adherence to Standards

FY 2017 evaluations were found to have high adherence to five of the 11 SOPs, moderate adherence to five of the standards, and low adherence to one of the standards (Table 3).

Reporting and adherence to standards improved in several ways between FY 2016 and FY 2017 (Figure 40). Country and regional teams reported an increase of 94 completed reports with checklists in FY 2016 compared with 109 in FY 2017. The total number of evaluations fully meeting an SOP (score = yes) increased for all standards. Improvements between FY 2016 and FY 2017, by percentage for a score of yes, occurred across all standards' adherence to the following:

- SOP 1 (stakeholder engagement) improved from 45 percent (42) to 57 percent (62)
- SOP 2 (evaluation purpose) improved from 72 percent (68) to 99 percent (108)
- SOP 3 (appropriate evaluation design, methods, and analytical techniques) improved from 89 percent (84) to 97 percent (106)
- SOP 4 (ethical considerations) improved from 74 percent (70) to 83 percent (90)
- SOP 5 (articulate budget) improved from 18 percent (17) to 23 percent (25)

Table 3: Adherence to Standards FY 2017

	SOP 1	SOP 2	SOP 3	SOP 4	SOP 5	SOP 6	SOP 7	SOP 8	SOP 9	SOP 10	SOP 11
Yes	62	108	106	90	25	107	50	59	86	63	52
Partial	4	0	2	3	27	1	39	6	0	20	39
No	43	1	1	16	57	1	20	44	23	26	18
% Adherence	57%	99%	97%	83%	23%	98%	46%	54%	79%	58%	48%

High (61% or greater) Moderate (41 - 60%) Low (40% or less)

[31] Results of the completed evaluation for HRSA were not included in the analysis below, but will be incorporated in future reporting.

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A mother with her child in Uganda.

- SOP 6 (data collection and management) improved from 94 percent (88) to 98 percent (107)
- SOP 7 (ensuring appropriate evaluator competencies and qualifications) improved from 17 percent (16) to 46 percent (50)
- SOP 8 (monitoring implementation) improved from 40 percent (38) to 54 percent (59)
- SOP 9 (produce quality reports) improved from 16 percent (15) to 79 percent (86)
- SOP 10 (dissemination) improved from 15 percent (14) to 58 percent (63)
- SOP 11 (use of findings) improved from 37 percent (35) to 48 percent (52)

The one standard that requires significant improvement is SOP 5 (articulate budget). While there were improvements, noted above, in many of the standards, SOP 5 is still showing low adherence at 23 percent. There are multiple reasons for poor adherence to this standard, one being that including budget data on the cost of evaluation, IS, and OR activities is not a standard practice and therefore has been a slow process to integrate.

Discussion

PEPFAR will continue providing field teams with technical assistance and support to improve areas that fall short of high adherence. Overall adherence scores are expected to increase in subsequent years as 1) ESOP will be used to inform all newly started evaluations, 2) implementing partners become more familiar with the standards and improve evaluation reporting, and 3) agencies work to amend policies so they adhere to ESOP.

PEPFAR will continue working closely with headquarters and country/regional teams to improve the quality of evaluations and expand the availability of results. Existing gaps are being actively analyzed to assess how to best fulfill existing policies and requirements, and whether any need special consideration or modification. PEPFAR is pursuing ways to increase the engagement of headquarters and country/regional-level staff with evaluators and working to promote these SOPs to all implementing partners and ensure improved adherence.

Agencies are integrating more formal evaluation requirements, tying the standards into contracts, and monitoring efforts as the number of evaluations conducted increase. These results also highlight the need to improve public dissemination of reports and findings to reach 100 percent. PEPFAR is reviewing agency policies and practices to ensure they are consistent and share the same ultimate objective of public access. This year, greater attention will be focused on more strategic

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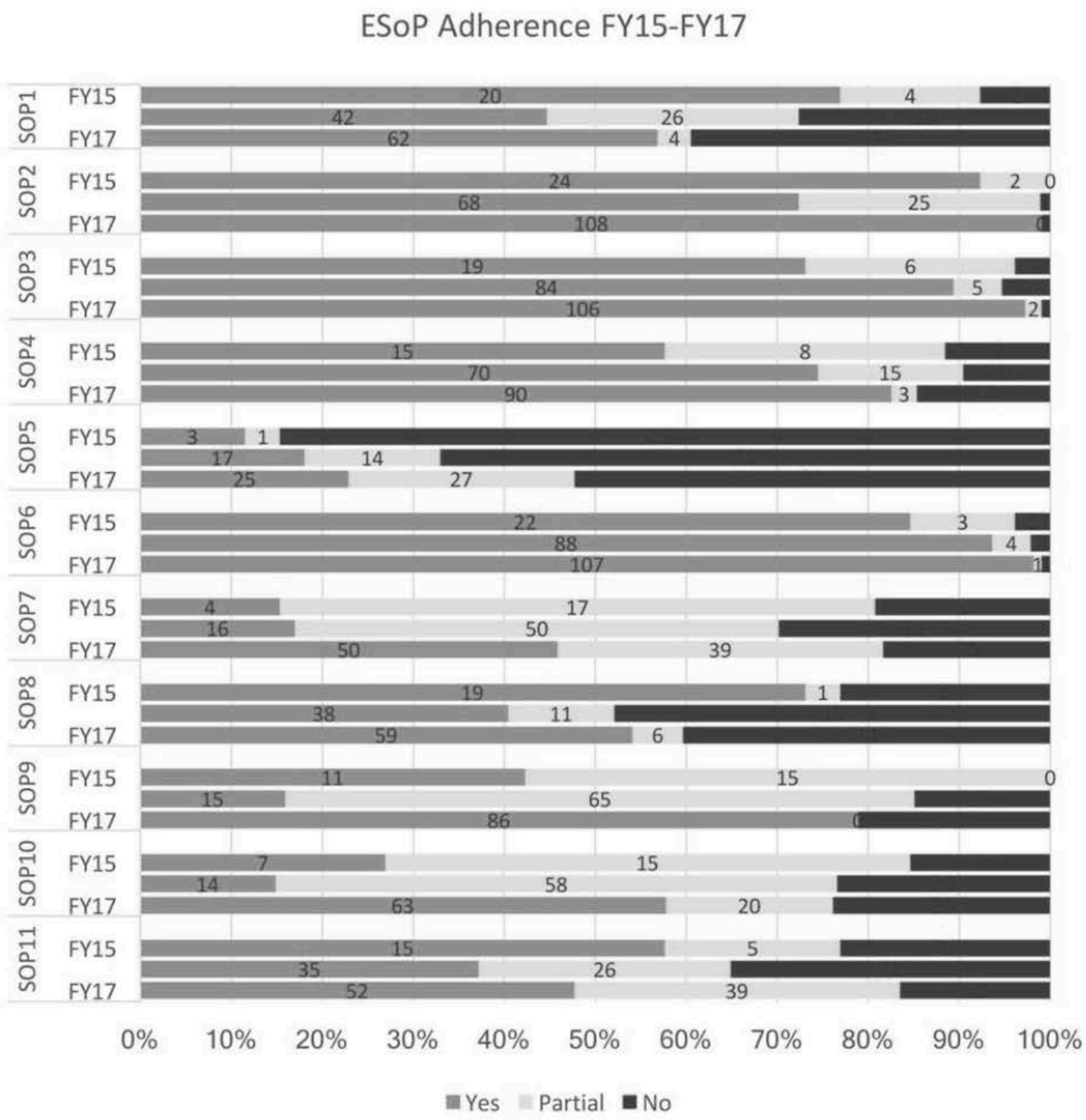
Figure 40: Report Adherence to Evaluation Standards of Practice, FY 2015–2017



evaluation portfolios that are well-planned, answer existing evidence gaps, and are linked to country/regional priorities and the PEPFAR goal of reaching the UNAIDS 90-90-90 targets.

PEPFAR's recently created Epidemic Control Teams (ECTs) will help emphasize the use of evidence-based programming to ensure that all PEPFAR countries and regions are investing in and scaling up interventions that are proven to be effective and efficient. The continued accurate and consistent reporting of evaluation data will help inform ECTs, the solutions they propose, and the identification of evidence gaps for which future investments would be prudent.

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GLOSSARY

ACT	Accelerating Children's HIV/AIDS Treatment Initiative
AGYW	Adolescent Girls and Young Women
ANC	Antenatal Care
ART	Antiretroviral Therapy/Treatment
ARV	Antiretroviral Medications
CDC	Centers for Disease Control and Prevention
COP	Country Operational Plan
CSC	Community Scorecard
CSO	Civil Society Organization
DREAMS	Determined, Resilient, Empowered, AIDS-free, Mentored and Safe
DTG	Dolutegravir
ECT	Epidemic Control Team
EJAF	Elton John AIDS Foundation
ESOP	Evaluation Standards of Practice
FBO	Faith-Based Organization
FY	Fiscal Year
GBV	Gender-Based Violence
GHCC	Global Health Cost Consortium
Global Fund	The Global Fund to Fight AIDS, Tuberculosis and Malaria
GPSDD	Global Partnership for Sustainable Development Data
GSD	Gender and Sexual Diversity
HBCU	Historically Black Colleges and Universities
HCW	Health Care Worker
HIV	Human Immunodeficiency Virus
HRH	Human Resources for Health
HRSA	Heath Resources and Services Administration
HTS	HIV Testing Services
JSI	JSI Research & Training Institute, Inc.
KP	Key Population
LCI	Local Capacity Initiative
LGBT	Lesbian, Gay, Bisexual, and Transgender
MER	Monitoring, Evaluation, and Reporting
MLP	Midlevel Practitioners
MOFs	Ministries of Finance
MOH	Minister of Health
MSI	Minority-Serving Institutions
MSM	Men who have Sex with Men
NGO	Non-Governmental Organization
OVC	Orphans and Vulnerable Children
PCB	Program Coordinating Board
PEPFAR	U.S. President's Emergency Plan for AIDS Relief
PHIA	Population-Based HIV/AIDS Impact Assessment
PLHIV	People Living with HIV
PMTCT	Prevention of Mother-to-Child Transmission
PPP	Public-Private Partnerships
PrEP	Pre-Exposure Prophylaxis
PRRR	Pink Ribbon Red Ribbon
ROP	Regional Operational Plan
SDS	Strategic Direction Summary
SFI	Sustainable Finance Initiative

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SHIMS	Swaziland HIV Incidence Measurement Surveys
SID	Sustainability Index and Dashboard
TB	Tuberculosis
TLD	Tenofovir/Lamivudine/Dolutegravir
UNAIDS	Joint United Nations Programme on HIV/AIDS
UN	United Nations
UNGA	United Nations General Assembly
USAID	United States Agency for International Development
VAC	Violence Against Children
VMMC	Voluntary Medical Male Circumcision
WHO	World Health Organization

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PEPFAR

U.S. President's Emergency Plan for AIDS Relief

U.S. Department of State
Office of the U.S. Global AIDS Coordinator
and Health Diplomacy

April 2018

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From: Folkers, Greg (NIH/NIAID) [E]
Sent: Mon, 7 May 2018 22:07:50 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: these

January 25, 2018 **PEPFAR — 15 Years and Counting the Lives Saved**
Article in *The New England Journal of Medicine*, co-authored by Dr. Robert W. Eisinger
January 19, 2018 **Ending the HIV/AIDS Pandemic**
Article in *Emerging Infectious Diseases*, co-authored by Dr. Robert W. Eisinger
June 6, 2016 **An AIDS-free world is possible, but do we have the will to achieve it?**
Commentary at STAT News, co-authored by Dr. Deborah L. Birx
January 10, 2016 **No more excuses. We have the tools to end the HIV/AIDS pandemic.**
Commentary in the Washington Post.
December 1, 2015 **Ending the HIV-AIDS Pandemic — Follow the Science**
Commentary in The New England Journal of Medicine, co-authored by Dr. Hilary Marston.
December 1, 2015 **Time to Write the Last Chapter on HIV** Commentary at CNN.com, co-authored by Dr. Chris Beyrer, President of the International AIDS Society.
January 27, 2015 **Focusing to Achieve a World Without AIDS** Commentary in The Journal of the American Medical Association, co-authored by Dr. Hilary D. Marston.

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Sent: Mon, 7 May 2018 16:51:26 +0000
To: Fauci, Anthony (NIH/NIAID) [E];Marston, Hilary (NIH/NIAID) [E]
Subject: HIV monoclonals meeting prebrief (Hilary)

Is it possible to get 30 minutes to prebrief for the 6/13 DAIDS workshop on Development of Monoclonal Antibodies for HIV Treatment and Cure? Late this week is fine, of course. Greg would be most welcome.

Many thanks,

Hilary

Hilary Marston, MD, MPH
Medical Officer and Policy Advisor for Global Health
NIAID Office of the Director
(b)(6)
(b)(6) (cell)

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From: Paules, Catharine (NIH/NIAID) [E]
Sent: Mon, 7 May 2018 12:12:52 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Accepted: Prebrief for Influenza immunology writing project

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Mon, 7 May 2018 12:10:06 +0000
To: Fauci, Anthony (NIH/NIAID) [E];Paules, Catharine (NIH/NIAID) [E]
Subject: Prebrief for Influenza immunology writing project

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Mon, 7 May 2018 12:10:06 +0000
To: Fauci, Anthony (NIH/NIAID) [E];Paules, Catharine (NIH/NIAID) [E]
Subject: Prebrief for Influenza immunology writing project

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Mon, 7 May 2018 10:11:38 +0000
To: 'Anthony Fauci'
Subject: FW: Harrison's Principles of Internal Medicine, 20e -- PAGE PROOFS ----- Chapter 197
Attachments: Chapter 197 page proof.pdf

From: Folkers, Greg (NIH/NIAID) [E]
Sent: Sunday, May 06, 2018 10:40 PM
To: Tyagi, Anupriya (b)(6)
Cc: Anthony Fauci (b)(6) (b)(6) (b)(6) Fauci, Anthony (NIH/NIAID) [E] (b)(6) Davis, Kim (b)(6)
Subject: RE: Harrison's Principles of Internal Medicine, 20e -- PAGE PROOFS ----- Chapter 197

Anupriya,
Attached are the page proofs for chapter 197, with minor corrections noted.

Best regards
G

From: Tyagi, Anupriya (b)(6)
Sent: Thursday, May 03, 2018 12:26 PM
To: Folkers, Greg (NIH/NIAID) [E] (b)(6) Lane, Cliff (NIH/NIAID) [E] (b)(6)
Cc: Anthony Fauci (b)(6) (b)(6) (b)(6) Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: RE: Harrison's Principles of Internal Medicine, 20e -- PAGE PROOFS ----- Chapter 197

Thanks so much!

Regards,
Anupriya

From: Folkers, Greg (NIH/NIAID) [E] (b)(6)
Sent: Thursday, May 3, 2018 9:30 PM
To: Tyagi, Anupriya; Lane, Cliff (NIH/NIAID) [E]
Cc: Anthony Fauci (b)(6) (b)(6) Fauci, Anthony (NIH/NIAID) [E]
Subject: RE: Harrison's Principles of Internal Medicine, 20e -- PAGE PROOFS ----- Chapter 197

Anupriya,
We found just a couple very minor things to fix
We are going over the revised tables once last time and will get to shortly.

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Greg

From: Tyagi, Anupriya (b)(6)
Sent: Thursday, May 03, 2018 3:25 AM
To: Folkers, Greg (NIH/NIAID) [E] (b)(6)
Cc: Anthony Fauci (b)(6) (b)(6) (b)(6)
Subject: RE: Harrison's Principles of Internal Medicine, 20e -- PAGE PROOFS ----- Chapter 197
Importance: High

Hi Dr. Folkers,

Just checking to see if you've reviewed the chapter and have any corrections. Please let me know as we're finalizing the chapters now.

Regards,
Anupriya

From: Tyagi, Anupriya
Sent: Wednesday, April 25, 2018 4:06 PM
To: (b)(6)
Cc: Anthony Fauci (b)(6) (b)(6)
Subject: Harrison's Principles of Internal Medicine, 20e -- PAGE PROOFS ----- Chapter 197

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Thanks!

Regards,
Anupriya

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cases, decreased spasticity in three, and restoration of the ability to walk after confinement to a wheelchair in two. Antibody to IL-15 receptor β chain has been tested with some promising clinical effects in small numbers of patients. Physical therapy and rehabilitation are important components of management.

FEATURES OF HTLV-2 INFECTION

Epidemiology HTLV-2 is endemic in certain Native American tribes and in Africa. It is generally considered to be a New World virus that was brought from Asia to the Americas 10,000–40,000 years ago during the migration of infected populations across the Bering land bridge. The mode of transmission of HTLV-2 is probably the same as that of HTLV-1 (see above). HTLV-2 may be less readily transmitted sexually than HTLV-1.

Studies of large cohorts of injection drug users with serologic assays that reliably distinguish HTLV-1 from HTLV-2 indicated that the vast majority of HTLV-positive cohort members were infected with HTLV-2. The seroprevalence of HTLV in a cohort of 7841 injection drug users from drug treatment centers in Baltimore, Chicago, Los Angeles, New Jersey (Asbury Park and Trenton), New York City (Brooklyn and Harlem), Philadelphia, and San Antonio was 20.9%, with >97% of cases due to HTLV-2. The seroprevalence of HTLV-2 was higher in the Southwest and the Midwest than in the Northeast. In contrast, the seroprevalence of HIV-1 was higher in the Northeast than in the Southwest or the Midwest. Approximately 3% of the cohort members were infected with both HTLV-2 and HIV-1. The seroprevalence of HTLV-2 increased linearly with age. Women were significantly more likely than men to be infected with HTLV-2; the virus is thought to be more efficiently transmitted from male to female than from female to male.

Associated Diseases Although HTLV-2 was isolated from a patient with a T-cell variant of hairy-cell leukemia, this virus has not been consistently associated with a particular disease and in fact has been thought of as "a virus searching for a disease." However, evidence is accumulating that HTLV-2 may play a role in certain neurologic, hematologic, and dermatologic diseases. These data require confirmation, particularly in light of the previous confusion regarding the relative prevalences of HTLV-1 and HTLV-2 among injection drug users.

Prevention Avoidance of needle sharing, adherence to safe-sex practices, screening of blood (by assays for HTLV-1, which also detect HTLV-2), and avoidance of breast-feeding by infected women are important principles in the prevention of spread of HTLV-2.

HUMAN IMMUNODEFICIENCY VIRUS

HIV-1 and HIV-2 are members of the lentivirus subfamily of Retroviridae and are the only lentiviruses known to infect humans. The lentiviruses are slower-acting than viruses that cause acute infection (e.g., influenza virus) but not than other retroviruses. The features of acute primary infection with HIV resemble those of more classic acute infections. The characteristic chronicity of HIV disease is consistent with the designation *lentivirus*. **For a detailed discussion of HIV, see Chap. 197.**

FURTHER READING

Ishida T et al: Multicenter phase II study of lenalidomide in relapsed or recurrent adult T-cell leukemia-lymphoma: ATLL-002. J Clin Oncol 34:4086, 2016.

Katsuya H et al: Treatment and survival among 1594 patients with ATL. Blood 126:2570, 2015.

Ma G et al: Multifaceted functions and roles of HBA in HTLV-1 pathogenesis. Retrovirology 13:16, 2016.

Moir S et al: Pathogenic mechanisms of HIV disease. Annu Rev Pathol 6:223, 2011.

Panfil AR et al: Human T-cell leukemia virus–associated malignancy. Curr Opin Virol 20:40, 2016.

197 Human Immunodeficiency Virus Disease: AIDS and Related Disorders

Anthony S. Fauci, Gregory K. Folkers, H. Clifford Lane

AIDS was first recognized in the United States in the summer of 1981, when the U.S. Centers for Disease Control and Prevention (CDC) reported the unexplained occurrence of *Pneumocystis jirovecii* (formerly *P. carinii*) pneumonia in five previously healthy homosexual men in Los Angeles and of Kaposi's sarcoma (KS) with or without *P. jirovecii* pneumonia and other opportunistic infections in 26 previously healthy homosexual men in New York, San Francisco, and Los Angeles. The disease was soon recognized in male and female injection drug users; in hemophiliacs and blood transfusion recipients; among female sexual partners of men with AIDS; and among infants born to mothers with AIDS. In 1983, human immunodeficiency virus (HIV) was isolated from a patient with lymphadenopathy, and by 1984 it was demonstrated clearly to be the causative agent of AIDS. In 1985, a sensitive enzyme-linked immunosorbent assay (ELISA) was developed; this led to an appreciation of the scope and evolution of the HIV epidemic at first in the United States and other developed nations and ultimately among developing nations throughout the world (see "HIV Infection and AIDS Worldwide," below). The staggering worldwide evolution of the HIV pandemic has been matched by an explosion of information in the areas of HIV virology, pathogenesis (both immunologic and virologic), treatment of HIV disease, treatment and prophylaxis of the opportunistic diseases associated with HIV infection, and prevention of HIV infection. The information flow related to HIV disease is enormous and continues to expand, and it has become almost impossible for the health care generalist to stay abreast of the literature. The purpose of this chapter is to present the most current information available on the scope of the pandemic; on its pathogenesis, treatment, and prevention; and on prospects for vaccine development. Above all, the aim is to provide a solid scientific basis and practical clinical guidelines for a state-of-the-art approach to the HIV-infected patient.

DEFINITION

The current U.S. CDC classification system for HIV infection and AIDS categorizes patients based on clinical conditions associated with HIV infection together with the level of the CD4+ T lymphocyte count. A confirmed HIV case can be classified in one of five HIV infection stages (0, 1, 2, 3, or unknown). If there was a negative HIV test within 6 months of the first HIV infection diagnosis, the stage is 0, and remains 0 until 6 months after diagnosis. Advanced HIV disease (AIDS) is classified as stage 3 if one or more specific opportunistic illness has been diagnosed **(Table 197-1)**. Otherwise, the stage is determined by CD4+ T lymphocyte test results and immunologic criteria **(Table 197-2)**. If none of these criteria apply (e.g., because of missing information on CD4+ T lymphocyte test results), the stage is U (unknown).

The definition and staging criteria of AIDS are complex and comprehensive and were established for surveillance purposes rather than for the practical care of patients. Thus, the clinician should not focus on whether the patient fulfills the strict definition of AIDS, but should view HIV disease as a spectrum ranging from primary infection, with or without the acute syndrome, to the relatively asymptomatic stage, to advanced stages associated with opportunistic diseases (see "Pathophysiology and Pathogenesis," below).

ETIOLOGIC AGENT

HIV is the etiologic agent of AIDS; it belongs to the family of human retroviruses (Retroviridae) and the subfamily of lentiviruses **(Chap. 196)**. Nononcogenic lentiviruses cause disease in other animal

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TABLE 197-1 CDC Stage 3 (AIDS)-Defining Opportunistic Illnesses in HIV Infection

Bacterial infections, multiple or recurrent[a]
Candidiasis of bronchi, trachea, or lungs
Candidiasis of esophagus
Cervical cancer, invasive[b]
Coccidioidomycosis, disseminated or extrapulmonary
Cryptococcosis, extrapulmonary
Cryptosporidiosis, chronic intestinal (>1 month's duration)
Cytomegalovirus disease (other than liver, spleen, or nodes), onset at age >1 month
Cytomegalovirus retinitis (with loss of vision)
Encephalopathy attributed to HIV
Herpes simplex: chronic ulcers (>1 month's duration) or bronchitis, pneumonitis, or esophagitis (onset at age >1 month)
Histoplasmosis, disseminated or extrapulmonary
Isosporiasis, chronic intestinal (>1 month's duration)
Kaposi's sarcoma
Lymphoma, Burkitt's (or equivalent term)
Lymphoma, immunoblastic (or equivalent term)
Lymphoma, primary, of brain
Mycobacterium avium complex or *Mycobacterium kansasii*, disseminated or extrapulmonary
Mycobacterium tuberculosis of any site, pulmonary,[b] disseminated, or extrapulmonary
Mycobacterium, other species or unidentified species, disseminated or extrapulmonary
Pneumocystis jirovecii (previously known as *Pneumocystis carinii*) pneumonia
Pneumonia, recurrent[b]
Progressive multifocal leukoencephalopathy
Salmonella septicemia, recurrent
Toxoplasmosis of brain, onset at age >1 month
Wasting syndrome attributed to HIV

[a]Only among children age <6 years. [b]Only among adults, adolescents, and children age ≥6 years.

Source: MMWR 63(RR-03), April 11, 2014.

species, including sheep, horses, goats, cattle, cats, and monkeys. The four retroviruses known to cause human disease belong to two distinct groups: the human T lymphotropic viruses (HTLV)-1 and HTLV-2, which are transforming retroviruses; and the human immunodeficiency viruses, HIV-1 and HIV-2, which cause cytopathic effects either directly or indirectly (Chap. 196). The most common cause of HIV disease throughout the world, and certainly in the United States, is HIV-1, which comprises several subtypes with different geographic distributions (see "Molecular Heterogeneity of HIV-1," below). HIV-2 was first identified in 1986 in West African patients and was originally confined to West Africa. However, cases traced to West Africa or to sexual contacts with West Africans have been identified throughout the world. The currently defined groups of HIV-1 (M, N, O, P) and the HIV-2 groups A through H each are likely derived from a separate transfer to humans from a nonhuman primate reservoir. HIV-1 viruses likely came from chimpanzees and/or gorillas, and HIV-2 from sooty mangabeys. The AIDS pandemic is primarily caused by the HIV-1 M group viruses. Although HIV-1 group O and HIV-2 viruses have been found in numerous countries, including those in the developed world, they have caused much more localized epidemics. The taxonomic relationship between primate lentiviruses is shown in Fig. 197-1.

TABLE 197-2 CDC HIV Infection Stages 1–3 Based on Age-Specific CD4+ T Lymphocyte Count or CD4+ T Lymphocyte Percentage of Total Lymphocytes[a]

	AGE ON DATE OF CD4 T+ LYMPHOCYTE TEST					
	<1 YEAR		1–5 YEARS		6 YEARS THROUGH ADULT	
STAGE[a]	CELLS/μL	%	CELLS/μL	%	CELLS/μL	%
1	≥1500	≥34	≥1000	≥30	≥500	≥26
2	750–1,499	26–33	500–999	22–29	200–499	14–25
3	<750	<26	<500	<22	<200	<14

[a]The stage is based primarily on the CD4+ T lymphocyte count; the CD4+ T lymphocyte count takes precedence over the CD4+ T lymphocyte percentage, and the percentage is considered only if the count is missing.

Source: MMWR 63(RR-03), April 11, 2014.

MORPHOLOGY OF HIV

Electron microscopy shows that the HIV virion is an icosahedral structure (Fig. 197-2) containing numerous external spikes formed by the two major envelope proteins, the external gp120 and the transmembrane gp41. The HIV envelope exists as a trimeric heterodimer. The virion buds from the surface of the infected cell (Fig. 197-2*A*) and incorporates a variety of host cellular proteins into its lipid bilayer. The structure of HIV-1 is schematically diagrammed in Fig. 197-2*B*.

REPLICATION CYCLE OF HIV

HIV is an RNA virus whose hallmark is the reverse transcription of its genomic RNA to DNA by the enzyme *reverse transcriptase*. The replication cycle of HIV begins with the high-affinity binding via surface-exposed residues within the gp120 protein to its receptor on the host cell surface, the CD4 molecule (Fig. 197-3). The CD4 molecule is a 55-kDa protein found predominantly on a subset of T lymphocytes that are responsible for helper function in the immune system (Chap. 342). Once it binds to CD4, the gp120 protein undergoes a conformational change that facilitates binding to one of two major co-receptors. The two major co-receptors for HIV-1 are CCR5 and CXCR4. Both receptors belong to the family of seven-transmembrane-domain G protein–coupled cellular receptors, and the use of one or the other or both receptors by the virus for entry into the cell is an important determinant of the cellular tropism of the virus. Cell-to-cell spread is also facilitated by accessory molecules such as the C-type lectin receptor *DC-SIGN* expressed on certain dendritic cells (DCs) that bind to the HIV gp120 envelope protein, allowing virus captured on DCs to spread to CD4+ T cells. Following binding of the envelope protein to the CD4 molecule associated with the above-mentioned conformational change in the viral envelope gp120, fusion with the host cell membrane occurs via the newly exposed gp41 molecule penetrating the plasma membrane of the target cell and then coiling upon itself to bring the virion and target cell together (Fig. 197-4). Following fusion, uncoating of the capsid protein shell is initiated—a step that facilitates reverse transcription and leads to formation of the preintegration complex, composed of viral RNA, enzymes, and accessory proteins and surrounded by capsid and matrix proteins (Fig. 197-3). As the preintegration complex traverses the cytoplasm to reach the nucleus, the viral reverse transcriptase enzyme catalyzes the reverse transcription of the genomic RNA into DNA, resulting in the formation of double-stranded proviral HIV DNA. At several steps of the replication cycle, the virus is vulnerable to various cellular factors that can block the progression of infection. The cytoplasmic tripartite motif-containing protein 5-α (TRIM5-α) is a host restriction factor that interacts with retroviral capsids, causing their premature disassembly and induction of innate immune responses. While early studies with laboratory strains found the HIV-1 capsid bound weakly to the human form of TRIM5-α, capsids of primary isolates appear to be more susceptible to TRIM5-α-mediated disassembly. The apolipoprotein B mRNA editing enzyme (catalytic polypeptide-like 3 [APOBEC3]) family of cellular proteins also inhibits progression of virus infection after virus has entered the cell and prior to entering the nucleus. APOBEC3 proteins, which are incorporated into virions and released into the cytoplasm of a newly infected cell, bind to the single minus-strand DNA intermediate and deaminate viral cytidine, causing hypermutation of retroviral genomes. HIV has evolved a powerful strategy to protect itself from APOBEC. The viral protein Vif targets

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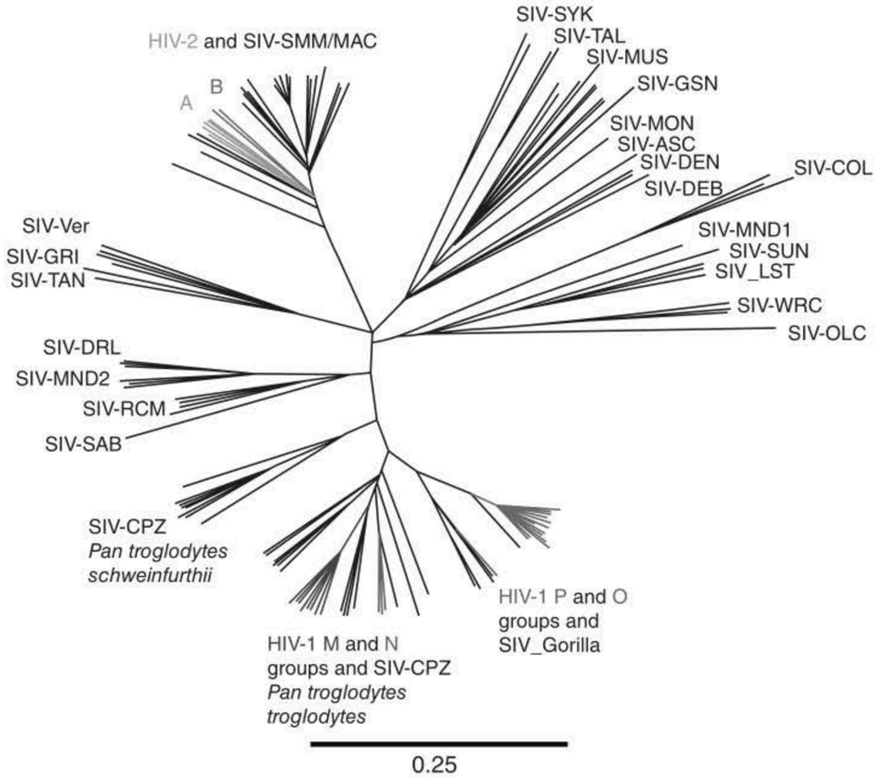

FIGURE 197-1 A phylogenetic tree based on the nearly complete genomes (*gag* through *nef* genes) of primate immunodeficiency viruses. The scale (0.25) indicates a 25% phylogenetically corrected genetic distance at the nucleotide level. Clades in color represent viruses (HIV-1, HIV-2) identified in humans after relatively recent transfers from chimpanzee, gorilla, and sooty mangabey reservoirs. *(Prepared by Brian Foley, PhD, of the HIV Sequence Database, Theoretical Biology and Biophysics Group, Los Alamos National Laboratory; additional information at www.hiv.lanl.gov/content/sequence/HelpDocs/subtypes.html.)*

APOBEC3 for proteasomal degradation. SAMHD1 is another post-entry host factor that prevents reverse transcription by depleting pools of deoxynucleotides (dNTPs). The type I interferon (IFN)-induced myxovirus resistance protein 2 (MX2) is another restriction factor associated with innate immunity that inhibits HIV-1 nuclear entry.

With activation of the cell, the viral DNA accesses the nuclear pore and is transferred from the cytoplasm to the nucleus, where it is integrated into the host cell chromosomes through the action of another virally encoded enzyme, *integrase* (Fig. 197-3). HIV proviral DNA integrates into the host genomic DNA preferentially in regions of active transcription and regional hotspots. This provirus may remain transcriptionally inactive (latent) or it may manifest varying levels of gene expression, up to active transcription and production of virus depending on the metabolic state of the infected cell.

Cellular activation plays an important role in the replication cycle of HIV and is critical to the pathogenesis of HIV disease (see "Pathogenesis and Pathophysiology," below). Following initial binding, fusion, and internalization of the nucleic acid contents of virions into the target cell, incompletely reverse-transcribed DNA intermediates are labile in quiescent cells and do not integrate efficiently into the host cell genome unless cellular activation occurs shortly after infection. Furthermore, some degree of activation of the host cell is required for the initiation of transcription of the integrated proviral DNA into either genomic RNA or mRNA. This latter process may not necessarily be associated with the detectable expression of the classic cell-surface markers of activation. In this regard, activation of HIV expression from the latent state depends on the interaction of a number of cellular and viral factors. Following transcription, HIV mRNA is translated into proteins that undergo modification through glycosylation, myristoylation, phosphorylation, and cleavage. The viral particle is formed by the assembly of HIV proteins, enzymes, and genomic RNA at the plasma membrane of the cells. Budding of the progeny virion through the lipid bilayer of the host cell membrane is the point at which the core acquires its external envelope and where the host restriction factor tetherin can inhibit the release of budding particles. Tetherin is an IFN-induced type II transmembrane protein that interferes with virion detachment, although the HIV accessory protein Vpu counteracts this effect through direct interactions with tetherin. During or soon after budding, the virally encoded protease catalyzes the cleavage of the gag-pol precursor to yield the mature virion. Progression through the virus replication cycle is profoundly influenced by a variety of viral regulatory gene products. Likewise, each point in the replication cycle of HIV is a real or potential target for therapeutic intervention. Thus far, the reverse transcriptase, protease, and integrase enzymes as well as the process of virus–target cell binding and fusion have proved to be susceptible to pharmacologic disruption.

HIV GENOME

Figure 197-5 illustrates schematically the arrangement of the HIV genome. Like other retroviruses, HIV-1 has genes that encode the structural proteins of the virus: *gag* encodes the proteins that form the core of the virion (including p24 antigen); *pol* encodes the enzymes responsible for protease processing of viral proteins, reverse transcription, and integration; and *env* encodes the envelope glycoproteins. However, HIV-1 is more complex than other retroviruses, particularly those of the nonprimate group, in that it also contains at least six other regulatory genes (*tat*, *rev*, *nef*, *vif*, *vpr*, and *vpu*), which code for proteins involved in the modification of the host cell to enhance virus growth and the regulation of viral gene expression. Several of these proteins are thought to play a role in the pathogenesis of HIV disease; their various functions are listed in Fig. 197-5. Flanking these genes are the long terminal repeats (LTRs), which contain regulatory elements involved in gene expression (Fig. 197-5). The major difference between the genomes of HIV-1 and HIV-2 is the fact that HIV-2 lacks the *vpu* gene and has a *vpx* gene not contained in HIV-1.

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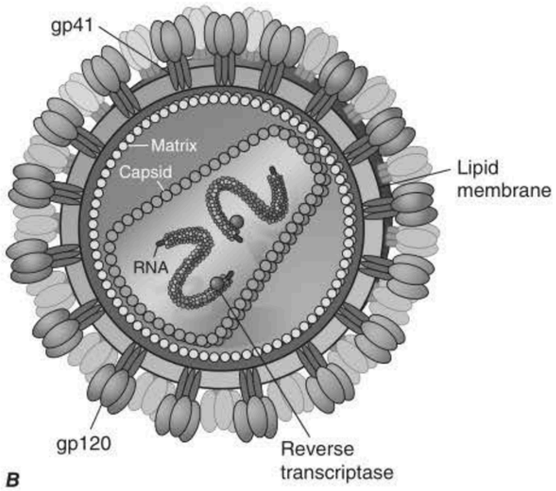
A



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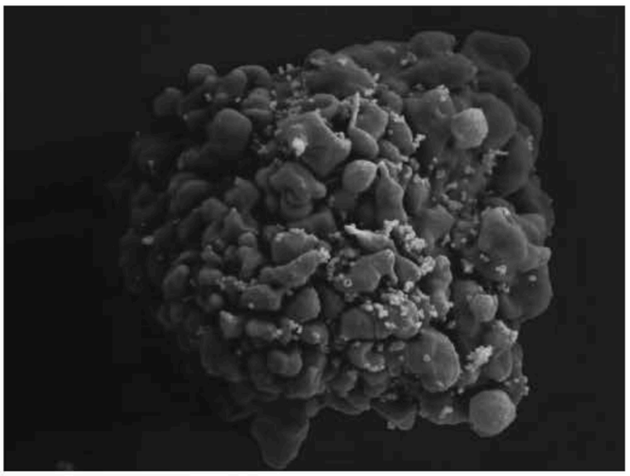

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FIGURE 197-2 ***A.*** Electron micrograph of HIV. Figure illustrates a typical virion following budding from the surface of a CD4+ T lymphocyte, together with two additional incomplete virions in the process of budding from the cell membrane. ***B.*** Structure of HIV-1, including the gp120 envelope, gp41 transmembrane components of the envelope, genomic RNA, enzyme reverse transcriptase, p18(17) inner membrane (matrix), and p24 core protein (capsid). *(Copyright by George V. Kelvin.) (Adapted from RC Gallo: Sci Am 256:46, 1987.)* ***C.*** Scanning electron micrograph of HIV-1 virions infecting a human CD4+ T lymphocyte. The original photograph was imaged at 20,000× magnification. Cell is approximately 10 microns in diameter, and the HIV particles are approximately 120 nanometers. *(Courtesy of Elizabeth R. Fischer, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases; with permission.)*

MOLECULAR HETEROGENEITY OF HIV-1

Molecular analyses of HIV isolates reveal varying levels of sequence diversity over all regions of the viral genome. For example, the degree of difference in the coding sequences of the viral envelope protein ranges from a few percent (very close, among isolates from the same infected individual) to more than 50% (extreme diversity, between isolates from the different groups of HIV-1: M, N, O, and P). The changes tend to cluster in hypervariable regions. HIV can evolve by several means, including simple base substitution, insertions and deletions, recombination, and gain and loss of glycosylation sites. HIV sequence diversity arises directly from the limited fidelity of the reverse transcriptase, i.e. a tendency toward copying errors. The balance of immune pressure and functional constraints on proteins influences the regional level of variation within proteins. For example, Envelope, which is exposed on the surface of the virion and is under immune selective pressure from both antibodies and cytolytic T lymphocytes, is extremely variable, with clusters of mutations in hypervariable domains. In contrast, reverse transcriptase, with important enzymatic functions, is relatively conserved, particularly around the active site. The extraordinary variability of HIV-1 contrasts markedly with the relative stability of HTLV-1 and 2.

The four groups (M, N, O and P) of HIV-1 are the result of four separate chimpanzee-to-human (or possibly gorilla-to-human for groups O and P) transfers. Group M (major), which is responsible for most of the infections in the world, has diversified into subtypes and intersubtype recombinant forms, due to "sub-epidemics" within humans after one of those transfers.

Among primate lentiviruses, HIV-1 is most closely related to viruses isolated from chimpanzees and gorillas (Fig. 197-1). The chimpanzee subspecies *Pan troglodytes troglodytes* has been established to be the natural reservoir of the HIV-1 M and N groups. The rare viruses of the HIV-1 O and P groups are most closely related to viruses found in Cameroonian gorillas. The M group comprises nine subtypes, or *clades*, designated A, B, C, D, F, G, H, J, and K, as well as more than 90 known circulating recombinant forms (CRFs) and numerous unique recombinant forms. Intersubtype recombinants are generated by infection of an individual with two subtypes that then recombine and create a virus with a selective advantage. These CRFs range from highly prevalent forms such as CRF01_AE, common in southeast Asia, and CRF02_AG from west and central Africa, to a large number of CRFs that are relatively rare, either because they are of a more recent origin (newly recombined) or because they have not broken out into a major population. The subtypes and CRFs create the major lineages of the M group of HIV-1. HIV-1 M group subtype C dominates the global pandemic, and although there is much speculation that it is more transmissible than other subtypes, solid data on variations in transmissibility

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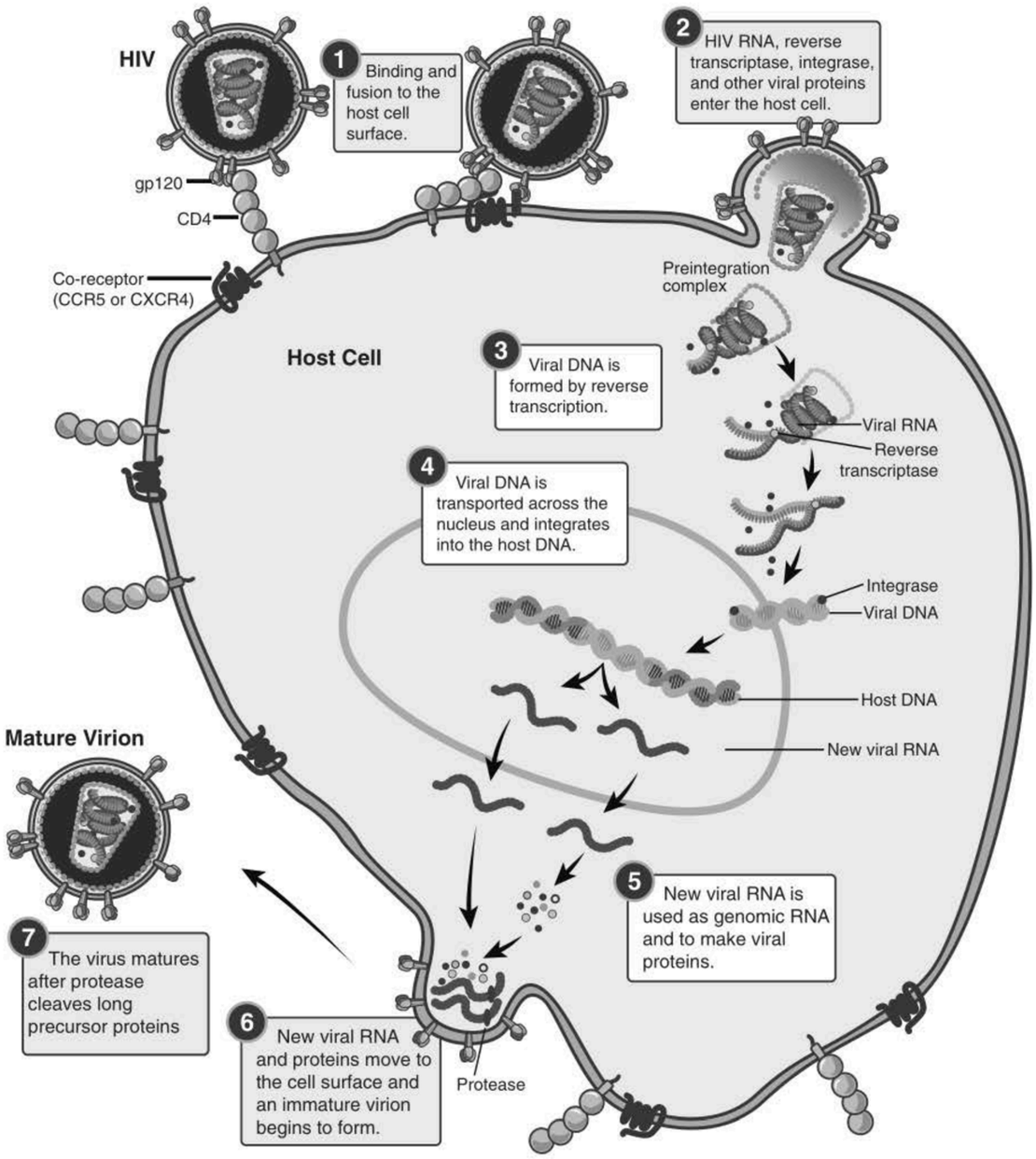

FIGURE 197-3 The replication cycle of HIV. See text for description. *(From the National Institute of Allergy and Infectious Diseases)*

between subtypes are lacking. Human population densities, access to prevention and treatment, prevalence of genital ulcers, iatrogenic transmissions, and other confounding host factors are all possible reasons why one subtype has spread more than another.

Figure 197-6 schematically diagrams the worldwide distribution of HIV-1 subtypes by region. Nine strains account for the vast majority of HIV infections globally: HIV-1 subtypes A, B, C, D, F, G and three of the CRFs, CRF01_AE, CRF02_AG, and CRF07_BC. Subtype C viruses (of the M group) are by far the most common form worldwide, likely accounting for ~50% of prevalent infections worldwide. In sub-Saharan Africa, home to approximately two-thirds of all individuals living with HIV/AIDS, most infections are caused by subtype C, with smaller proportions of infections caused by subtype A, subtype D, CRF02_AG, and other subtypes and recombinants. In South Africa, the country with the largest number of prevalent infections (7.1 million in 2016), >98% of the HIV-1 isolates sequenced are of subtype C. In Asia, HIV-1 isolates of the CRF01_AE lineage and subtypes B and C predominate. CRF01_AE accounts for most infections in south and southeast Asia, while >95% of infections in India, home to an estimated 2.1 million HIV-infected individuals, are of subtype C (see "HIV Infection and AIDS Worldwide," below). Subtype B viruses are the overwhelmingly predominant viruses seen in the United States, Canada, certain countries in South America, western Europe, and Australia. It is thought that, purely by chance, subtype B was seeded into the United States and Europe in the late 1970s, thereby establishing an overwhelming founder effect. Many countries have co-circulating viral subtypes that are giving rise to new CRFs. Sequence analyses of HIV-1 isolates from infected individuals indicate that recombination among viruses of different clades likely occurs when an individual is infected with viruses of more than one subtype, particularly in geographic areas where subtypes overlap, and more often in sub-epidemics driven by IV drug use than in those driven by sexual transmission.

The extraordinary diversity of HIV, reflected by the presence of multiple subtypes, circulating recombinant forms, and continuous viral evolution, has implications for possible differential rates of transmission, rates of disease progression, and the development of resistance to antiretroviral drugs. This diversity may also prove to be a formidable obstacle to HIV vaccine development, as a broadly useful vaccine would need to induce protective responses against a wide range of viral strains.

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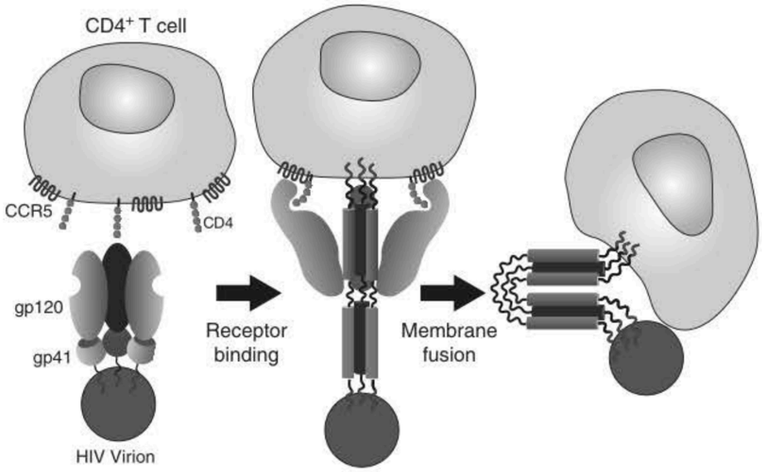

FIGURE 197-4 Binding and fusion of HIV-1 with its target cell. HIV-1 binds to its target cell via the CD4 molecule, leading to a conformational change in the gp120 molecule that allows it to bind to the co-receptor CCR5 (for R5-using viruses). The virus then firmly attaches to the host cell membrane in a coiled-spring fashion via the newly exposed gp41 molecule. Virus-cell fusion occurs as the transitional intermediate of gp41 undergoes further changes to form a hairpin structure that draws the two membranes into close proximity (see text for details). *(Adapted from D Montefiori, JP Moore: Science 283:336, 1999; with permission.)*

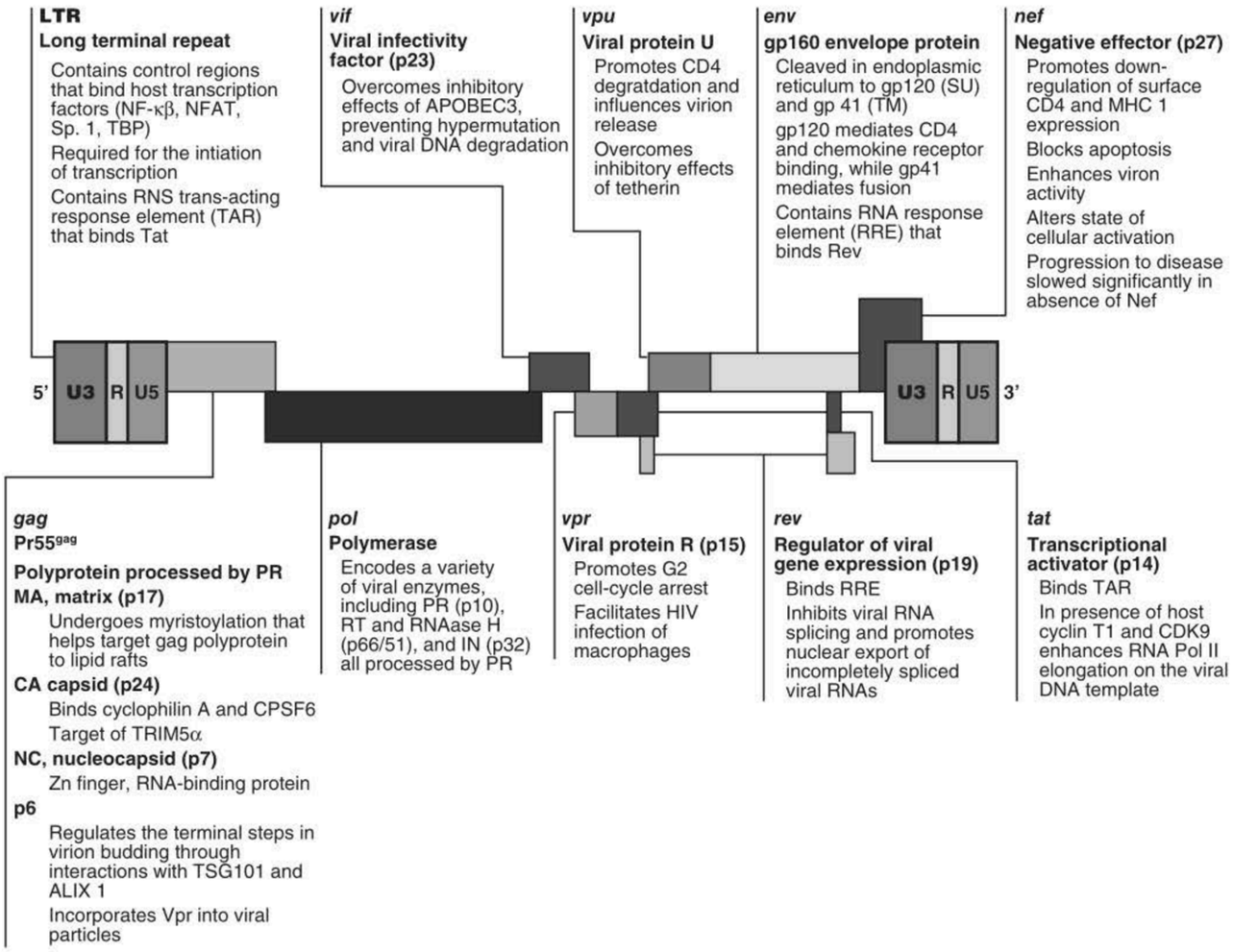

FIGURE 197-5 Organization of the genome of the HIV provirus together with a summary description of its 9 genes encoding 15 proteins. *(Adapted from WC Greene, BM Peterlin: Nat Med 8:673, 2002.)*

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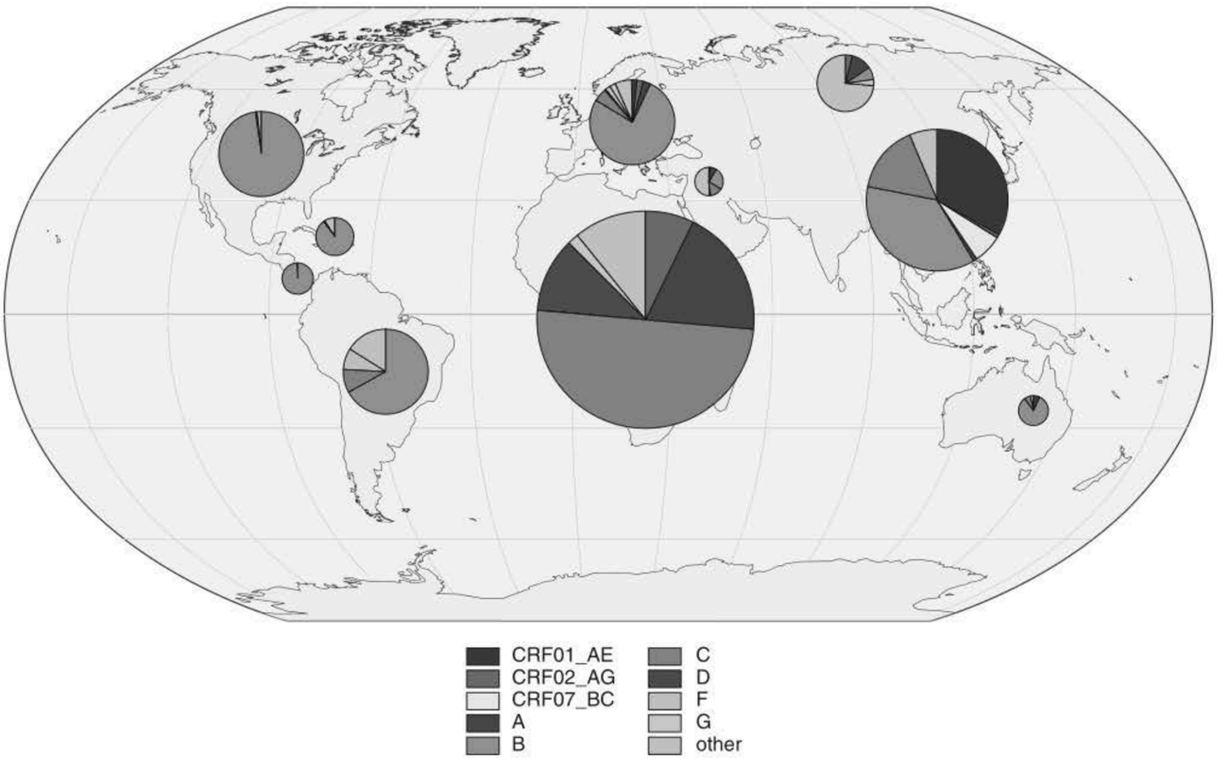

FIGURE 197-6 Global geographic distribution of HIV-1 subtypes and recombinant forms. Distributions derived from relative frequency of subtypes among >710,000 HIV genomic sequences in the Los Alamos National Laboratory HIV Sequence Database. (Additional information available at *www.hiv.lanl.gov/components/sequence/HIV/geo/geo.comp*.)

TRANSMISSION

HIV is transmitted primarily by sexual contact (both heterosexual and male to male); by blood and blood products; and by infected mothers to infants intrapartum, perinatally, or via breast milk. After more than 35 years of experience and observations, there is no evidence that HIV is transmitted by any other modality. Table 197-3 lists the estimated risk of HIV transmission for various types of exposures.

TABLE 197-3 Estimated Per-Act Probability of Acquiring HIV From an Infected Source, By Exposure Act

TYPE OF EXPOSURE	RISK PER 10,000 EXPOSURES
Parenteral	
Blood transfusion	9250
Needle-sharing during injection drug use	63
Percutaneous (needle-stick)	23
Sexual	
Receptive anal intercourse	138
Insertive anal intercourse	11
Receptive penile-vaginal intercourse	8
Insertive penile-vaginal intercourse	4
Receptive oral intercourse	Low
Insertive oral intercourse	Low
Other[a]	
Biting	Negligible
Spitting	Negligible
Throwing body fluids (including semen or saliva)	Negligible
Sharing sex toys	Negligible

[a]HIV transmission through these exposure routes is technically possible but unlikely and not well documented.

Sources: CDC, *www.cdc.gov/hiv/risk/estimates/riskbehaviors.html*; P Patel: AIDS 28:1509, 2014.

SEXUAL TRANSMISSION

HIV infection is predominantly a sexually transmitted infection (STI) worldwide. By far the most common mode of infection, particularly in developing countries, is heterosexual transmission, although in many western countries male-to-male sexual transmission dominates. Although a wide variety of factors including viral load and the presence of ulcerative genital diseases influence the efficiency of heterosexual transmission of HIV, such transmission is generally inefficient. A recent systemic review found a low per-act risk of heterosexual transmission in the absence of antiretrovirals: 0.04% for female-to-male transmission and 0.08% for male-to-female transmission during vaginal intercourse in the absence of antiretroviral therapy or condom use (Table 197-3).

HIV has been demonstrated in seminal fluid both within infected mononuclear cells and in cell-free material. The virus appears to concentrate in the seminal fluid, particularly in situations where there are increased numbers of lymphocytes and monocytes in the fluid, as seen in genital inflammatory states such as urethritis and epididymitis, conditions closely associated with other STIs. The virus has also been demonstrated in cervical smears and vaginal fluid. There is an elevated risk of HIV transmission associated with unprotected receptive anal intercourse (URAI) among both men and women compared to the risk associated with unprotected receptive vaginal intercourse. Although data are limited, the per-act risk for HIV transmission via URAI has been estimated to be ~1.4% (Table 197-3). The risk of HIV acquisition associated with URAI is higher than that seen in penile-vaginal intercourse probably because only a thin, fragile rectal mucosal membrane separates the deposited semen from potentially susceptible cells in and beneath the mucosa, and micro-trauma of the mucosal membrane has been associated with anal intercourse. Anal douching and sexual practices that traumatize the rectal mucosa also increase the likelihood of infection. It is likely that anal intercourse provides at least two modalities of infection: (1) direct inoculation into blood in cases of traumatic tears in the mucosa; and (2) infection of susceptible target

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cells, such as Langerhans cells, in the mucosal layer in the absence of trauma. Insertive anal intercourse also confers an increased risk of HIV acquisition compared to insertive vaginal intercourse in the receptive partner since the vaginal mucosa is several layers thicker than the rectal mucosa and less likely to be traumatized during intercourse. Nonetheless, the virus can be transmitted to either partner through vaginal intercourse. As noted in Table 197-3, male-to-female HIV transmission is more efficient than female-to-male transmission. The differences in reported transmission rates between men and women may be due in part to the prolonged exposure to infected seminal fluid of the vaginal and cervical mucosa, as well as the endometrium (when semen enters through the cervical os). By comparison, the penis and urethral orifice of the uninfected male partner are exposed relatively briefly to infected vaginal fluid. Among various cofactors examined in studies of heterosexual HIV transmission, the presence of other STIs has been strongly associated with HIV transmission. In this regard, there is a close association between genital ulcerations and transmission, owing to both susceptibility to infection and infectivity. Infections with microorganisms such as *Treponema pallidum* **(Chap. 177)**, *Haemophilus ducreyi* **(Chap. 152)**, and herpes simplex virus (HSV; **Chap. 187**) are important causes of genital ulcerations linked to transmission of HIV. In addition, pathogens responsible for non-ulcerative inflammatory STIs such as those caused by *Chlamydia trachomatis* **(Chap. 184)**, *Neisseria gonorrhoeae* **(Chap. 151)**, and *Trichomonas vaginalis* **(Chap. 224)** also are associated with an increased risk of transmission of HIV infection. Bacterial vaginosis, an infection related to sexual behavior, but not strictly an STI, also may be linked to an increased risk of transmission of HIV infection. Several studies suggest that treating other STIs and genital tract syndromes may help prevent transmission of HIV. This effect is most prominent in populations in which the prevalence of HIV infection is relatively low. It is noteworthy that this principle may not apply to the treatment of HSV infections since it has been shown that even following anti-HSV therapy with resulting healing of HSV-related genital ulcers, HIV acquisition is not reduced. Biopsy studies revealed that the likely explanation is that HIV receptor–positive inflammatory cells persisted in the genital tissue despite the healing of ulcers, and so HIV-susceptible targets remained at the site.

The quantity of HIV-1 in plasma (viral load) is a primary determinant of the risk of HIV-1 transmission. In a cohort of heterosexual couples in Uganda discordant for HIV infection and not receiving antiretroviral therapy, the mean serum HIV RNA level was significantly higher among HIV-infected subjects whose partners seroconverted than among those whose partners did not seroconvert. In fact, transmission was rare when the infected partner had a plasma level of <1700 copies of HIV RNA per milliliter, even when genital ulcer disease was present **(Fig. 197-7)**. The rate of HIV transmission per coital act was highest during the early stage of HIV infection when plasma HIV RNA levels were high and in advanced disease with high viral set points.

Antiretroviral therapy dramatically reduces plasma viremia in most HIV-infected individuals (see "Treatment," below) and is associated with a dramatic reduction in risk of transmission. In a large study of serodiscordant couples, earlier treatment of the HIV-infected partner with antiretroviral therapy rather than treatment delayed until the CD4+ T cell counts fell below 250 cells per μL was associated with a 96% reduction in HIV transmission to the uninfected partner. This approach has been widely referred to as *treatment as prevention* or TasP. Recent cohort studies have indicated that if the viral load of the infected partner is decreased to below detectable levels by antiretroviral therapy, there is essentially no chance of sexual transmission to the uninfected partner.

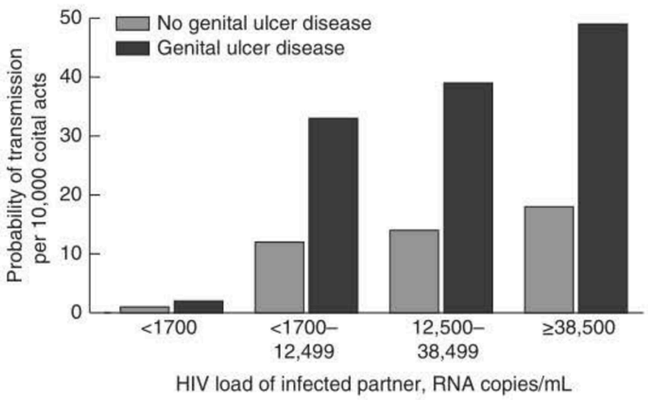

FIGURE 197-7 Probability of HIV transmission per coital act among monogamous, heterosexual, HIV-serodiscordant couples in Uganda. *(From RH Gray et al: Lancet 357:1149, 2001.)*

A number of studies including large, randomized, controlled trials clearly have indicated that male *circumcision* is associated with a lower risk of acquisition of HIV infection for heterosexual men. Studies also suggest that circumcision is protective in those men who have sex with men who are insertive only. The benefit of circumcision may be due to increased susceptibility of uncircumcised men to ulcerative STIs, as well as to other factors such as microtrauma to the foreskin and glans penis. In addition, the highly vascularized inner layer of foreskin tissue contains a high density of Langerhans cells as well as increased numbers of CD4+ T cells, macrophages, and other cellular targets for HIV. Finally, the moist environment under the foreskin may promote the presence or persistence of microbial flora that, via inflammatory changes, may lead to even higher concentrations of target cells for HIV in the foreskin. In addition, randomized clinical trials have demonstrated that male circumcision also reduces herpes simplex virus (HSV) type 2, human papillomavirus virus (HPV), and genital ulcer disease in men as well as HPV, genital ulcer disease, bacterial vaginosis, and *Trichomonas vaginalis* infections among female partners of circumcised men. Thus, there may be an added indirect benefit of diminution of risk for HIV acquisition to the female sexual partners of circumcised men.

In some studies the use of oral contraceptives was associated with an increase in incidence of HIV infection over and above that which might be expected by not using a condom for birth control. This phenomenon may be due to drug-induced changes in the cervical mucosa, rendering it more vulnerable to penetration by the virus. Adolescent girls might also be more susceptible to infection upon exposure due to the properties of an immature genital tract with increased cervical ectopy or exposed columnar epithelium.

Oral sex is a much less efficient mode of transmission of HIV than is anal intercourse or vaginal intercourse (Table 197-3). A number of studies have reported that the incidence of transmission of infection by oral sex among couples discordant for HIV was extremely low. However, there have been well-documented reports of HIV transmission that likely resulted from fellatio or cunnilingus. Therefore, the assumption that oral sex is completely safe is not warranted.

The association of alcohol consumption and illicit drug use with unsafe sexual behavior, both homosexual and heterosexual, leads to an increased risk of sexual transmission of HIV. Methamphetamine and other so-called club drugs (e.g., MDMA, ketamine, and gamma hydroxybutyrate), sometimes taken in conjunction with PDE-5 inhibitors such as sildenafil (Viagra), tadalafil (Cialis), or vardenafil (Levitra), have been associated with risky sexual practices and increased risk of HIV infection, particularly among men who have sex with men.

TRANSMISSION THROUGH INJECTION DRUG USE

HIV can be transmitted to injection drug users (IDUs) who are exposed to HIV while sharing injection paraphernalia such as needles, syringes, the water in which drugs are mixed, or the cotton through which drugs are filtered. Parenteral transmission of HIV during injection drug use does not require IV puncture; subcutaneous ("skin popping") or intramuscular ("muscling") injections can transmit HIV as well, even though these behaviors are sometimes erroneously perceived as low-risk. Among IDUs, the risk of HIV infection increases with the duration of injection drug use; the frequency of needle sharing; the number of partners with whom paraphernalia are shared, particularly in the setting of "shooting galleries" where drugs are sold and large numbers of IDUs may share a limited number of "works"; comorbid psychiatric conditions such as antisocial personality disorder; the use

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of cocaine in injectable form or smoked as "crack"; and the use of injection drugs in a geographic location with a high prevalence of HIV infection. As noted in Table 197-3, the per-act risk of transmission from injection drug use with a contaminated needle has been estimated to be approximately 0.6%.

TRANSMISSION BY TRANSFUSED BLOOD AND BLOOD PRODUCTS

HIV can be transmitted to individuals who receive HIV-contaminated blood transfusions, blood products, or transplanted tissue. The vast majority of HIV infections acquired via contaminated blood transfusions, blood components, or transplanted tissue in resource-rich countries occurred prior to the spring of 1985, when mandatory testing of donated blood for HIV-1 was initiated. It is estimated that >90% of individuals exposed to HIV-contaminated blood products become infected (Table 197-3). Transfusions of whole blood, packed red blood cells, platelets, leukocytes, and plasma are all capable of transmitting HIV infection. In contrast, hyperimmune gamma globulin, hepatitis B immune globulin, plasma-derived hepatitis B vaccine, and Rh_o immune globulin have not been associated with transmission of HIV infection. The procedures involved in processing these products either inactivate or remove the virus.

Currently, in the United States and in most developed countries, the following measures have made the risk of transmission of HIV infection by transfused blood or blood products extremely small: the screening of blood donations for antibodies to HIV-1 and HIV-2 and determination of the presence of HIV nucleic acid usually in minipools of several specimens; the careful selection of potential blood donors with health history questionnaires to exclude individuals with risk behavior; and opportunities for self-deferral and the screening out of HIV-negative individuals with serologic testing for infections that have shared risk factors with HIV, such as hepatitis B and C and syphilis. The chance of infection of a hemophiliac via clotting factor concentrates has essentially been eliminated because of standard screening of blood together with the added layer of safety resulting from heat treatment of the concentrates. It is currently estimated that the risk of infection with HIV in the United States via transfused screened blood is approximately 1 in 1.5 million units. Therefore, since nearly 21 million blood components are transfused in the United States each year, despite the best efforts of science, one cannot completely eliminate the risk of transfusion-related transmission of HIV. In this regard, a case of transfusion-related transmission of HIV was reported in the United States in 2010, which was tracked to a blood donation in 2008; this was the first such reported case since 2002 and only the third in that decade. Transmission of HIV (both HIV-1 and HIV-2) by blood or blood products is still an ongoing threat in certain developing countries where routine screening of blood is not universally practiced. In 2013, 108 out of 167 countries (65%) had specific legislation covering the safety and quality of blood transfusion, including 79% of high-income countries, 64% of middle-income countries, and 41% of low-income countries. Furthermore, there have been reports in certain countries of sporadic breakdowns in routinely available screening procedures in which contaminated blood was allowed to be transfused, resulting in small clusters of patients becoming infected.

OCCUPATIONAL TRANSMISSION OF HIV: HEALTH CARE WORKERS, LABORATORY WORKERS, AND THE HEALTH CARE SETTING

There is a small but definite occupational risk of HIV transmission to health care workers and laboratory personnel and potentially others who work with HIV-containing materials, particularly when sharp objects are used. An estimated 600,000 to 800,000 health care workers are stuck with needles or other sharp medical instruments in the United States each year. The global number of HIV infections among health care workers attributable to sharps injuries has been estimated to be 1000 cases (range, 200–5000) per year. In the United States, 58 documented cases of occupational HIV transmission to health care workers, and 150 possible transmissions have been reported by the CDC. There have been no confirmed cases reported since 1999.

Exposures that place a health care worker at potential risk of HIV infection are percutaneous injuries (e.g., a needle stick or cut with a sharp object) or contact of mucous membrane or nonintact skin (e.g., exposed skin that is chapped, abraded, or afflicted with dermatitis) with blood, tissue, or other potentially infectious body fluids. Large, multi-institutional studies have indicated that the risk of HIV transmission following skin puncture from a needle or a sharp object that was contaminated with blood from a person with documented HIV infection is ~0.23% and after a mucous membrane exposure it is 0.09% (see "HIV and the Health Care Worker," below) if the injured and/or exposed person is not treated within 24 h with antiretroviral drugs. The risk of hepatitis B virus (HBV) infection following a similar type of exposure is ~6–30% in nonimmune individuals; if a susceptible worker is exposed to HBV, postexposure prophylaxis with hepatitis B immune globulin and initiation of HBV vaccine is >90% effective in preventing HBV infection. The risk of HCV infection following percutaneous injury is ~1.8% **(Chap. 332)**.

Rare HIV transmission after nonintact skin exposure has been documented, but the average risk for transmission by this route has not been precisely determined; however, it is estimated to be less than the risk for mucous membrane exposure. Transmission of HIV through intact skin has not been documented. Currently in developed countries, virtually all puncture wounds and mucous membrane exposures in health care workers involving blood from a patient with documented HIV infection are treated prophylactically with combination antiretroviral therapy (cART). This practice, referred to as *postexposure prophylaxis* or PEP, has dramatically reduced the occurrence of puncture-related transmissions of HIV to health care workers.

In addition to blood and visibly bloody body fluids, semen and vaginal secretions also are considered potentially infectious; however, they have not been implicated in occupational transmission from patients to health care workers. The following fluids also are considered potentially infectious: cerebrospinal fluid, synovial fluid, pleural fluid, peritoneal fluid, pericardial fluid, and amniotic fluid. The risk for transmission after exposure to fluids or tissues other than HIV-infected blood also has not been quantified, but it is probably considerably lower than the risk after blood exposures. Feces, nasal secretions, saliva, sputum, sweat, tears, urine, and vomitus are not considered potentially infectious for HIV unless they are visibly bloody. Rare cases of HIV transmission via human bites have been reported, but not in the setting of occupational exposure.

An increased risk for HIV infection following percutaneous exposures to HIV-infected blood is associated with exposures involving a relatively large quantity of blood, as in the case of a device visibly contaminated with the patient's blood, a procedure that involves a hollow-bore needle placed directly in a vein or artery, or a deep injury. Factors that might be associated with mucocutaneous transmission of HIV include exposure to an unusually large volume of blood and prolonged contact. In addition, the risk increases for exposures to blood from untreated patients with high levels of HIV in the blood. Since the beginning of the HIV epidemic, there have been rare instances where transmission of infection from a health care worker to patients seemed highly probable. Despite these small number of documented cases, the risk of HIV transmission involving health care workers (infected or not) to patients is extremely low in developed countries—in fact, too low to be measured accurately. In this regard, several retrospective epidemiologic studies have been performed tracing thousands of patients of HIV-infected dentists, physicians, surgeons, obstetricians, and gynecologists, and no other cases of HIV transmission that could be linked to the health care providers were identified.

Breaches in infection control and the reuse of contaminated syringes, failure to properly sterilize surgical instruments, and/or hemodialysis equipment also have resulted rarely in the transmission of HIV from patient to patient in hospitals, nursing homes, and outpatient settings. Finally, these very rare occurrences of transmission of HIV as well as HBV and HCV to and from health care workers in the workplace underscore the importance of the use of universal precautions when caring for all patients (see below and **Chap. 137**).

AMERICAN OVERSIGHT

MOTHER-TO-CHILD TRANSMISSION OF HIV

HIV infection can be transmitted from an infected mother to her fetus during pregnancy, during delivery, or by breast-feeding. This remains an important form of transmission of HIV infection in some developing countries. Virologic analyses of aborted fetuses indicate that HIV can be transmitted to the fetus during the first or second trimesters of pregnancy. However, maternal transmission to the fetus occurs most commonly in the perinatal period. Two studies performed in Rwanda and the Democratic Republic of Congo (then called Zaire) indicated that among all mother-to-child transmissions of HIV, the relative proportions were 23–30% before birth, 50–65% during birth, and 12–20% via breast-feeding.

In the absence of antiretroviral therapy for the mother during pregnancy, labor, and delivery, and for the fetus prophylactically following birth, the probability of transmission of HIV from mother to infant/fetus ranges from 15% to 25% in industrialized countries and from 25% to 35% in developing countries. These differences may relate to the adequacy of prenatal care as well as to the stage of HIV disease and the general health of the mother during pregnancy. Higher rates of transmission have been reported to be associated with many factors—the best documented of which is the presence of high maternal levels of plasma viremia, with the risk increasing linearly with the level of maternal plasma viremia. It is very unlikely that mother-to-child transmission will occur if the mother's level of plasma viremia is <1000 copies of HIV RNA/mL of blood and extremely unlikely if the level is undetectable (i.e., <50 copies/mL). However, there may not be a lower "threshold" below which transmission never occurs, since certain studies have reported rare transmission by women with viral RNA levels <50 copies/mL. Increased mother-to-child transmission is also correlated with closer human leukocyte antigen (HLA) match between mother and child. A prolonged interval between membrane rupture and delivery is another well-documented risk factor for transmission. Other conditions that are potential risk factors, but that have not been consistently demonstrated, are the presence of chorioamnionitis at delivery; STIs during pregnancy; illicit drug use during pregnancy; cigarette smoking; preterm delivery; and obstetric procedures such as amniocentesis, amnioscopy, fetal scalp electrodes, and episiotomy. Today, the rate of mother-to-child transmission has fallen to 1% or less in pregnant women who are receiving cART for their HIV infection. Such treatment, combined with cesarean section delivery, has rendered mother-to-child transmission of HIV an unusual event in the United States and other developed nations. In this regard, both the United States Public Health Service and the World Health Organization guidelines recommend that all pregnant HIV-infected women should receive life-long cART for the health of the mother and to prevent perinatal transmission regardless of plasma HIV RNA copy number or CD4+ T cell counts.

Breast-feeding is an important modality of transmission of HIV infection in certain developing countries, particularly where mothers continue to breast-feed for prolonged periods. The risk factors for mother-to-child transmission of HIV via breast-feeding include detectable levels of HIV in breast milk, the presence of mastitis, low maternal CD4+ T cell counts, and maternal vitamin A deficiency. The risk of HIV infection via breast-feeding is highest in the early months of breast-feeding. In addition, exclusive breast-feeding has been reported to carry a lower risk of HIV transmission than mixed feeding. In developed countries, breast feeding of babies by an HIV-infected mother is contraindicated since alternative forms of adequate nutrition, i.e., formulas, are readily available. In developing countries, where breast-feeding may be essential for the overall health of the infant, the continuation of cART in the infected mother during the period of breastfeeding markedly diminishes the risk of transmission of HIV to the infant. In fact, once cART has been initiated in a pregnant woman, it should be continued for life.

TRANSMISSION OF HIV BY OTHER BODY FLUIDS

Although HIV can be isolated typically in low titers from saliva of a small proportion of infected individuals, there is no convincing evidence that saliva can transmit HIV infection, either through kissing or through other exposures, such as occupationally to health care workers. Saliva contains endogenous antiviral factors; among these factors, HIV-specific immunoglobulins of IgA, IgG, and IgM isotypes are detected readily in salivary secretions of infected individuals. It has been suggested that large glycoproteins such as mucins and thrombospondin 1 sequester HIV into aggregates for clearance by the host. In addition, a number of soluble salivary factors inhibit HIV to various degrees in vitro, probably by targeting host cell receptors rather than the virus itself. Perhaps the best studied of these, secretory leukocyte protease inhibitor (SLPI), blocks HIV infection in several cell culture systems, and it is found in saliva at levels that approximate those required for inhibition of HIV in vitro. In this regard, higher salivary levels of SLPI in breast-fed infants were associated with a decreased risk of HIV transmission through breast milk. It has also been suggested that submandibular saliva reduces HIV infectivity by stripping gp120 from the surface of virions, and that saliva-mediated disruption and lysis of HIV-infected cells occurs because of the hypotonicity of oral secretions. There have been outlier cases of suspected transmission by saliva, but these have probably been blood-to-blood transmissions. Transmission of HIV by a human bite can occur but is a rare event. Although virus can be identified, if not isolated, from virtually any body fluid, there is no evidence that HIV transmission can occur as a result of exposure to tears, sweat, or urine. However, there have been isolated cases of transmission of HIV infection by body fluids that may or may not have been contaminated with blood. Most of these situations occurred in the setting of a close relative providing intensive nursing care for an HIV-infected person without observing universal precautions, underscoring the importance of adhering to such precautions in the handling of body fluids and wastes from HIV-infected individuals.

EPIDEMIOLOGY

HIV INFECTION AND AIDS WORLDWIDE

HIV infection/AIDS is a global pandemic, with cases reported from virtually every country. At the end of 2016, an estimated 36.7 million individuals were living with HIV infection, according to the Joint United Nations Programme on HIV/AIDS (UNAIDS). An estimated 95% of people living with HIV/AIDS reside in low- and middle-income countries; ~50% are female, and 2.1 million are children <15 years. The regional distribution of these cases is illustrated in **Fig. 197-8**. The estimated number of people living with HIV—i.e., the global prevalence—has increased more than fourfold since 1990, reflecting the combined effects of continued high rates of new HIV infections and the life-prolonging impact of antiretroviral therapy (**Fig. 197-9**). In 2016, the global prevalence of HIV infection among people aged 15–49 years was 0.8%, with rates varying widely by country and region as illustrated in **Fig. 197-10**.

In 2016, an estimated 1.8 million new cases of HIV infection occurred worldwide, including 160,000 among children <15 years; about one-third of new infections were among people age 15–24 years. Globally, the majority of new HIV infections are due to heterosexual transmission. Members of certain high-risk populations are disproportionately affected. Sex workers, people who inject drugs, transgender people, prisoners, gay men, other men who have sex with men, and their sexual partners accounted for 34% of all new HIV infections in 2015 (**Fig. 197-11**).

Between 2000 and 2016, the estimated annual number of new HIV infections globally fell by 40% (Fig. 197-9). These reductions in global HIV incidence likely reflect progress with HIV prevention efforts and the increased provision to HIV-infected people of antiretroviral therapy, which makes them much less likely to transmit the virus to sexual partners. Among adults, the estimated incidence declined by 11% from 2010 to 2016. From 2010 to 2016 there was a ~47% reduction in HIV infections among children <15 years, progress that is due largely to the increasing availability of antiretroviral medications to prevent the transmission of HIV from mother to infant.

In 2016, global AIDS deaths totaled 1.0 million (including 120,000 children <15 years), a 48% decrease since 2005 that coincides with a

AMERICAN OVERSIGHT

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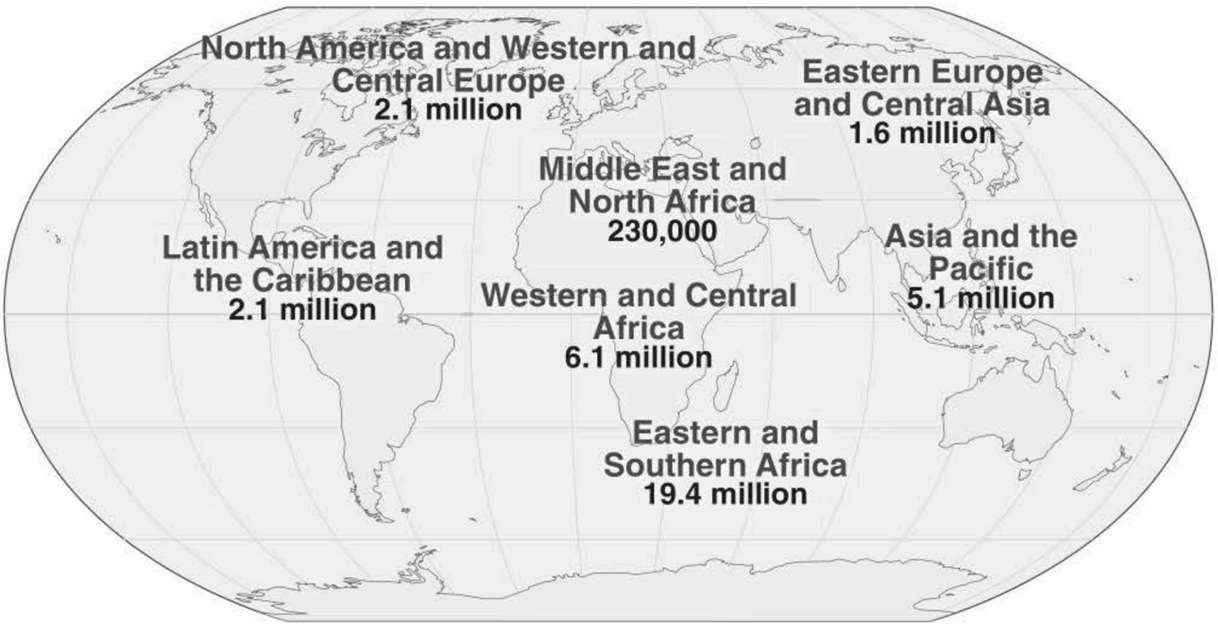

FIGURE 197-8 Estimated number of adults and children living with HIV infection as of December, 2016. Total: 36.7 million (30.8 million-42.9 million). *(From Joint United Nations Programme on HIV/AIDS [UNAIDS].)*

rapid expansion of access to antiretroviral therapy (Fig. 197-12). Since the beginning of the pandemic, an estimated 35 million people have died of an AIDS-related illness.

The HIV epidemic has occurred in "waves" in different regions of the world, each wave having somewhat different characteristics depending on the demographics of the country and region in question and the timing of the introduction of HIV into the population. Although the AIDS epidemic was first recognized in the United States and shortly thereafter in Western Europe, it very likely began in sub-Saharan Africa (see above), which has been particularly devastated by the epidemic. East and Southern Africa is the region hardest hit by HIV. The region is home to 6.2% of the world's population but has 19.4 million people living with HIV, >50% of the global total (Fig. 197-8). In eight countries in the region, >10% of the adult population age 15–49 is HIV-infected (Fig. 197-10). South Africa has the highest number of people living with HIV in the world (7.1 million); Swaziland has the highest adult HIV prevalence in the world (27.2%). Among high-risk individuals, rates are much higher than in the general population. HIV prevalence among sex workers varies between 50% and 70% in several countries in the region. Recent data offer promising signs of declining HIV incidence and prevalence in many countries in the region, although frequently at levels that remain high. Heterosexual exposure is the primary mode of HIV transmission in most countries in sub-Saharan Africa. Women and girls account for ~60 percent of all HIV infections in that region.

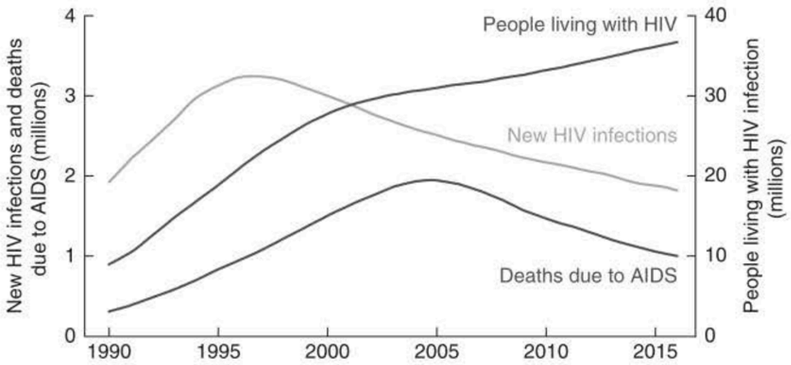

FIGURE 197-9 Global estimates of HIV incidence, AIDS deaths, and HIV prevalence 1990–2016. *(From UNAIDS.)*

The 25 countries of West and Central Africa are home to 6.1 million people living with HIV, of whom half a million are children. HIV prevalence in most of the countries is relatively low compared with East and Southern Africa. HIV prevalence among adults across the region overall stands at 2.2% although there is wide variation between countries, ranging from 0.5% in Niger and Senegal to 4.9% in Equatorial Guinea. An estimated 60% of new infections in the region in 2015 occurred in Nigeria. As in East and Southern Africa, heterosexual transmission accounts for most HIV transmission West and Central Africa.

The Middle East and North Africa region has one of the lowest HIV prevalence rates in the world (0.1%). In 2016, an estimated 230,000 people were living with HIV in the region. Cases are largely concentrated among IDUs, men who have sex with men, and sex workers and their clients.

In Asia and the Pacific, an estimated 5.1 million people were living with HIV at the end of 2016. In this region of the world, HIV prevalence is highest in southeast Asian countries, with wide variation in trends between different countries. Among countries in Asia, only Thailand has an adult seroprevalence rate of >1%. However, the populations of many Asian nations are so large that even low infection and seroprevalence rates result in large numbers of people living with HIV. In this regard, three populous countries—China, India and Indonesia—account for around three-quarters of all people living with HIV in the region. Although the HIV epidemic in Asia has long been concentrated among specific populations—sex workers and their clients, men who have sex with men, and IDUs—it is expanding to the heterosexual partners of those most at risk.

Eastern Europe and Central Asia is the only region in the world where the HIV epidemic continues to expand rapidly, with a >60% increase in annual new HIV infections between 2010 and 2016. The Russian Federation and Ukraine account for the majority of HIV cases in the region, where the epidemic has been driven by injection drug use and increasingly by heterosexual transmission.

Approximately 2.1 million people were living with HIV/AIDS in Latin America and the Caribbean at the end of 2016. The rate of new HIV infections in the region held steady from 2010 to 2016. Brazil is home to the largest number of HIV-infected persons (830,00) in the region, and the Bahamas has the region's highest prevalence (3.3%). Men who have sex with men

AMERICAN OVERSIGHT

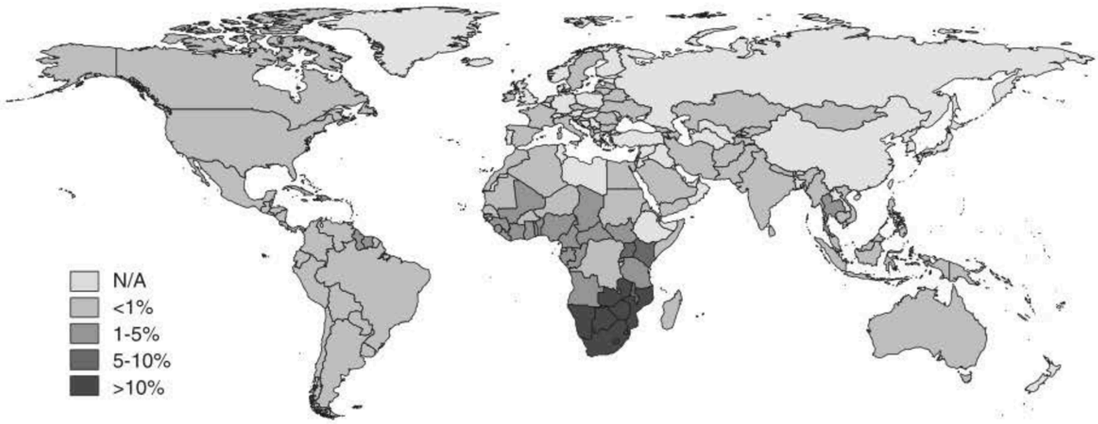

FIGURE 197-10 Adult HIV prevalence rates by country, 2016. Data are estimates for adults age 15–49 years. *(From UNAIDS.)*

account for the largest proportion of HIV infections in Central and South America. In the Caribbean, heterosexual transmission, often tied to sex work, is the main driver of transmission.

Approximately 2.1 million people were living with HIV/AIDS in North America and western and central Europe at the end of 2016. While modes of transmission vary greatly by country, HIV disproportionately affects men who have sex with men. Over the past decade, the number of HIV diagnoses decreased dramatically in all risk groups in western Europe but increased slightly in central Europe. North America saw a decrease in HIV diagnoses overall but a small increase among gay and bisexual men.

HIV INFECTION AND AIDS IN THE UNITED STATES

About 1.8 million people have been infected with HIV in the United States since the beginning of the epidemic, of whom ~693,000 have died. Approximately 1.1 million individuals in the United States are living with HIV infection, ~15% of whom are unaware of their infection, according to recent estimates. As illustrated in **Fig. 197-13**, only about half of HIV-infected people in the United States have been able to negotiate the steps in the HIV "care continuum," from diagnosis, to entering into care and receiving antiretroviral therapy, and ultimately to achieving a suppressed viral load (see "Treatment," below).

More than 60% of people living with HIV in the United States are Black/African-American or Hispanic/Latino, and more than half are men who have sex with men. The estimated HIV seroprevalence rate among all individuals age 13 years or older in the United States is ~0.5%. Approximately 2% of Black/African-American adults are HIV-infected in the United States, higher than any other group.

The number of new HIV infections in the United States, *HIV incidence*, peaked at about 130,000 per year in the late 1980s, followed by declines. After remaining stable since the mid-1990s, the estimated number of annual HIV infections in the United States fell ~15% between 2008 and 2015 (from 45,200 to 38,500). The distribution of incident HIV cases in 2015 is shown in **Fig. 197-14**. Gay and bisexual men account for more than two-thirds of incident infections and were the only group that did not experience an overall decline in annual HIV infections from 2008 to 2015. While infections among white gay and bisexual men and men age 15–24 years fell during that period, these declines were offset by increases among 25- to 34-year-old gay and bisexual men, and among Hispanic/Latino gay and bisexual males.

In the United States, the burden of HIV and AIDS is not evenly distributed across states and regions. In most areas of the country, HIV is concentrated in urban areas. In the southern United States, larger percentages of diagnoses are in smaller metropolitan and nonmetropolitan areas. HIV infection and AIDS have disproportionately affected minority populations in the United States in both urban and rural areas. Among those diagnosed with HIV (regardless of stage of infection) in 2016, 44% percent were Blacks/African Americans, a group that constitutes only 12% of the U.S. population. The estimated rate of new HIV diagnoses in 2016 by race/ethnicity per 100,000 population in the United States is shown in **Fig. 197-15**.

Perinatal HIV transmission, from an HIV-infected mother to her baby, has declined significantly in the United States, largely due to the implementation of guidelines for the universal counseling and voluntary HIV testing of pregnant women and the use of antiretroviral therapy for pregnant women and newborn infants to prevent infection. In 2016, 122 children were newly diagnosed with HIV infection in the United States, down from a peak of ~1750 in 1991.

The rate of HIV-related deaths in the United States rose steadily through the 1980s and peaked in 1995. Since then, the HIV death rate has fallen fourfold (**Fig. 197-16**). This trend is likely due to several factors, including improved prophylaxis and treatment of opportunistic infections, growing experience among the health professions in caring for HIV-infected individuals, improved access to health care, and a decrease in new infections. However, the most influential factor clearly has been the increased use of potent antiretroviral drugs, generally administered in a combination of three or four agents.

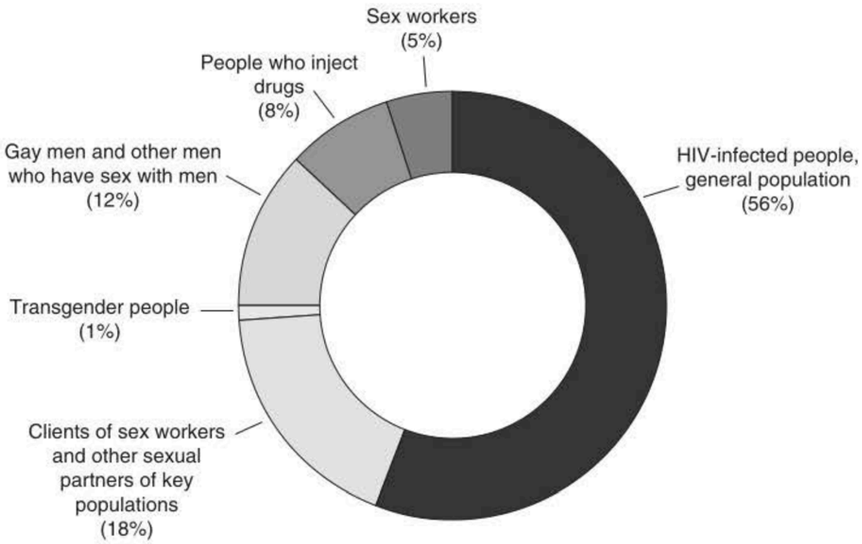

FIGURE 197-11 Global distribution of new HIV infections by population. Data for 2015. *(From UNAIDS).*

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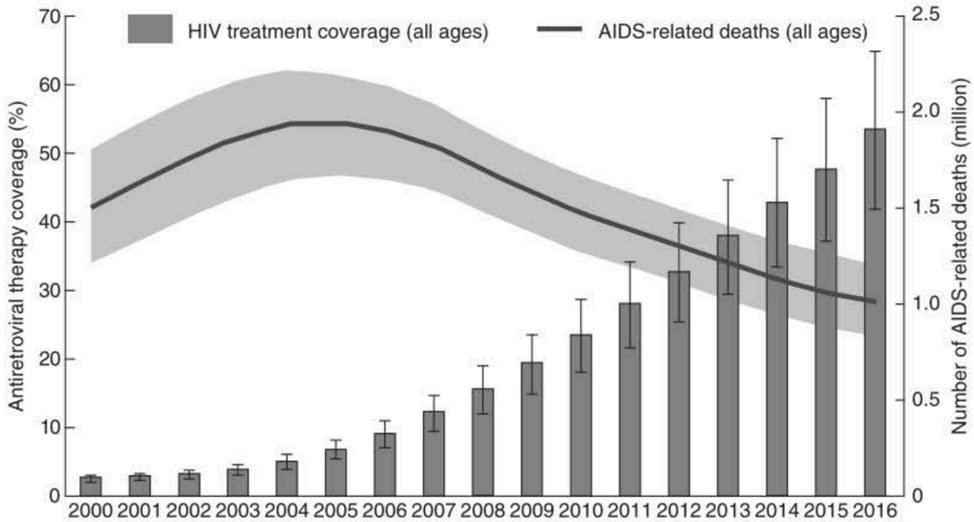

FIGURE 197-12 Global antiretroviral therapy coverage and number of AIDS-related deaths, 2000–2016. *(From UNAIDS).*

PATHOPHYSIOLOGY AND PATHOGENESIS

The hallmark of HIV disease is a profound immunodeficiency resulting primarily from a progressive quantitative and qualitative deficiency of the subset of T lymphocytes referred to as *helper T cells* occurring in a setting of polyclonal immune activation. The *helper* subset of T cells is defined phenotypically by the presence on its surface of the CD4 molecule (Chap. 342), which serves as the primary cellular receptor for HIV. A co-receptor also must be present together with CD4 for efficient binding, fusion, and entry of HIV-1 into its target cells (Figs. 197-3 and 197-4). HIV-1 uses two major co-receptors, CCR5 and CXCR4, for fusion and entry; these co-receptors are also the primary receptors for certain chemoattractive cytokines termed *chemokines* and belong to the seven-transmembrane-domain G protein–coupled family of receptors. A number of mechanisms responsible for cellular depletion and/or immune dysfunction of CD4+ T cells have been demonstrated in vitro; these include direct infection and destruction of these cells by HIV, as well as indirect effects such as immune clearance of infected cells, cell death associated with aberrant immune activation, and immune exhaustion due to aberrant cellular activation with resulting cellular dysfunction. Patients with CD4+ T cell levels below certain thresholds are at high risk of developing a variety of opportunistic diseases, particularly the infections and neoplasms that are AIDS-defining illnesses. Some features of AIDS, such as Kaposi's sarcoma and certain neurologic abnormalities, cannot be explained completely by the immunodeficiency caused by HIV infection, since these complications may occur prior to the development of severe immunologic impairment.

The combination of viral pathogenic and immunopathogenic events that occurs during the course of HIV disease from the moment of initial (primary) infection through the development of advanced-stage disease is complex and varied. It is important to appreciate that the pathogenic mechanisms of HIV disease are multifactorial and multiphasic and are different at different stages of the disease. Therefore, it is essential to consider the typical clinical course of an untreated HIV-infected individual in order to more fully appreciate these pathogenic events (Fig. 197-17).

EARLY EVENTS IN HIV INFECTION: PRIMARY INFECTION AND INITIAL DISSEMINATION OF VIRUS

Using rectal or vaginal mucosal transmission in nonhuman primates as a model, the earliest events (within hours) that occur following exposure of HIV to the mucosal surface determine whether an infection will be established or aborted as well as the subsequent course of events following infection. Although the mucosal barrier is relatively effective in limiting access of HIV to susceptible targets in the submucosal tissue, the virus can cross the barrier by transport on Langerhans cells, an epidermal type of DC, just beneath the surface or through microscopic rents in the mucosa. Significant disruptions in the mucosal barrier as seen in ulcerative genital disease facilitate viral entry and increase the efficiency of infection. Virus then seeks susceptible targets, which are primarily CD4+ T cells that are spatially dispersed in the mucosa. This spatial dispersion of targets provides a significant obstacle to the establishment of infection. Such obstacles account for the low efficiency of sexual transmission of HIV (see "Sexual Transmission," above). Both "partially" resting CD4+ T cells and activated CD4+ T cells serve as early amplifiers of infection. Resting CD4+ T cells are more abundant; however, activated CD4+ T cells produce larger amounts of virus. In order for infection to become established, the basic reproductive rate (R_0) must become equal to or greater than 1, i.e., each infected cell would infect at least one other cell. Once infection is established, the virus replicates in lymphoid cells in the mucosa, the submucosa, and to some extent the lymphoreticular tissues that drain the gut or genital tissues. For a variable period

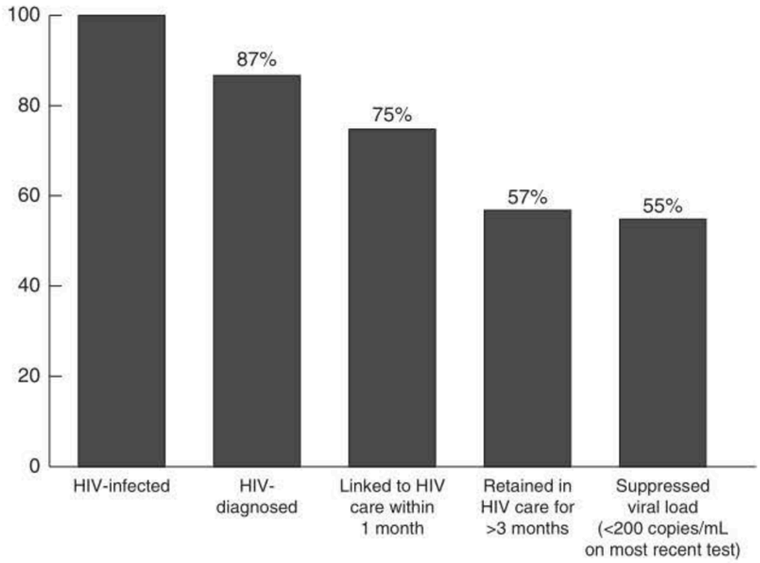

FIGURE 197-13 Estimated percentage of HIV-infected people engaged at selected stages of the continuum of HIV care in the United States. *(From Centers for Disease Control and Prevention [CDC]: HIV Surveillance Supplemental Report 21[No. 7], 2016.)*

AMERICAN OVERSIGHT

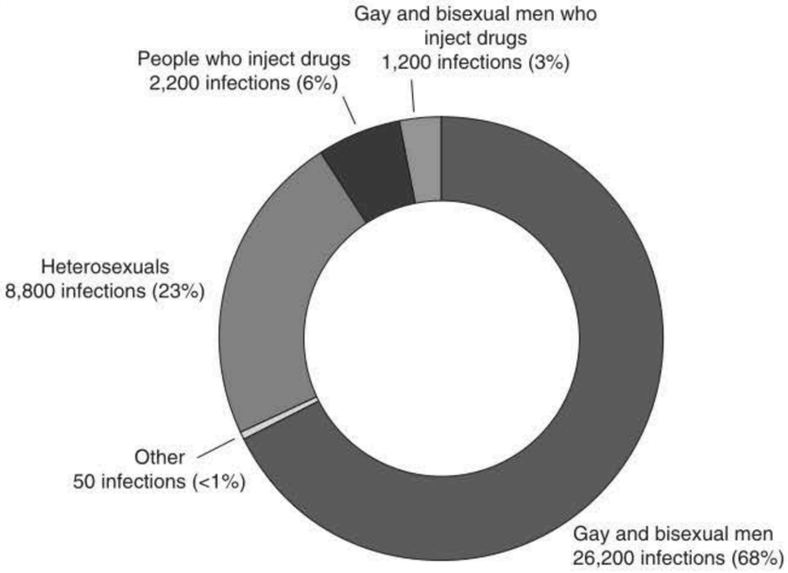

FIGURE 197-14 Estimated distribution of new HIV infections in the United States. Total: 38,500. Incidence estimate for 2015. *(From CDC.)*

of time ranging from a few to several days, the virus cannot yet be detected in the plasma. This period is referred to as the "eclipse" phase of infection. As more virus is produced within several days to weeks, it is disseminated, first to the draining lymph nodes and then to other lymphoid compartments where it has easy access to dense concentrations of CD4+ T cell targets, allowing for a burst of high-level viremia that is readily detectable by currently available assays (Fig. 197-18). The gut-associated lymphoid tissue (GALT), is a major target of HIV infection and the location where large numbers of CD4+ T cells (usually memory cells) are infected and depleted, both by direct viral effects and by activation-associated apoptosis. Once virus replication reaches this threshold and virus is widely disseminated, infection is firmly established throughout the lymphoid tissues of the body and the process is irreversible. It is important to point out that the initial infection of susceptible cells may vary somewhat with the route of infection. Virus that enters directly into the bloodstream via infected blood or blood products (i.e., transfusions, use of contaminated needles for injection drugs, sharp-object injuries, maternal-to-fetal transmission either intrapartum or perinatally, or sexual intercourse where there is enough trauma to cause bleeding) is likely first cleared from the circulation to the spleen and other lymphoid organs, where primary focal infections begin, followed by wider dissemination throughout other lymphoid tissues as described above.

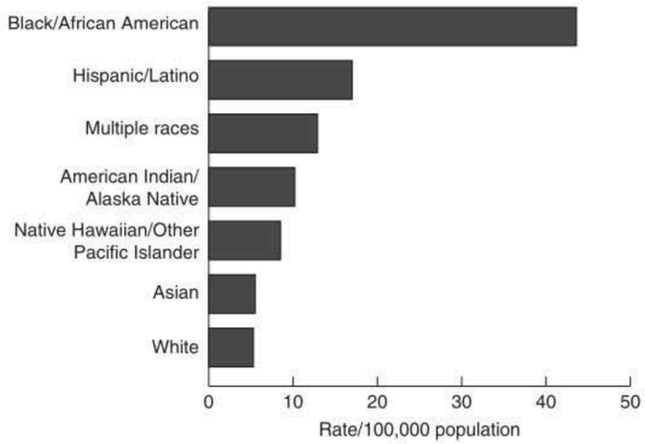

FIGURE 197-15 Estimated rate of HIV infections (including children) diagnosed during 2016 in the United States, by race/ethnicity (per 100,000 population). *(From CDC.)*

It has been demonstrated that sexual transmission of HIV is the result of a single infectious event and that a viral genetic bottleneck exists for transmission with selective transmission of certain viruses. In this regard, certain characteristics of the HIV envelope glycoprotein have a major influence on transmission, at least in subtype A and C viruses. Transmitting viruses, often referred to as "founder viruses," are usually underrepresented in the circulating viremia of the transmitting partner and are less-diverged viruses with signature sequences including shorter V1–V2 loop sequences and fewer predicted N-linked glycosylation sites relative to the major circulating variants. These viruses are almost exclusively R5 strains and are usually sensitive to neutralizing antibody. Once replication proceeds in the newly infected partner, the founder virus diverges and accumulates glycosylation sites, becoming progressively more resistant to neutralization (Fig. 197-19).

The acute burst of viremia and wide dissemination of virus in primary HIV infection may be associated with an *acute HIV syndrome*, which occurs to varying degrees in ~50% of individuals within 2 to 4 weeks of initial infection (see below). This syndrome is usually associated with high levels of viremia measured in millions of copies of HIV RNA per milliliter of plasma that last for several weeks. Acute mononucleosis-like symptoms are well correlated with the presence of viremia. Virtually all patients develop some degree of viremia during primary infection, which contributes to virus dissemination throughout the lymphoid tissue, even though they may remain asymptomatic or not recall experiencing symptoms. The initial level of plasma viremia in primary HIV infection does not necessarily determine the rate of disease progression; however, the set point of the level of steady-state plasma viremia after ~1 year seems to correlate with the slope of disease progression in the untreated patient. The strikingly high levels of viremia observed in many patients with acute HIV infection is felt to be associated with a higher likelihood of transmission of the virus to others by a variety of routes including sexual transmission, shared needles and syringes, and mother-to-child transmission intrapartum, perinatally, or via breast milk.

ESTABLISHMENT OF CHRONIC AND PERSISTENT INFECTION

Persistence of Virus Replication HIV infection is unique among human viral infections. Despite the robust cellular and humoral immune responses that are mounted following primary infection (see "Immune Response to HIV," below), once infection has been established the virus succeeds in escaping complete immune-mediated clearance, paradoxically seems to thrive on immune activation, and is never eliminated completely from the body. Rather, a chronic infection develops and persists with varying degrees of continual virus replication in the untreated patient for a median of ~10 years before the patient becomes clinically ill (see "Advanced HIV Disease," below). It is this establishment of a chronic, persistent infection that is the hallmark of HIV disease. Throughout the often-protracted course of chronic infection, virus replication can invariably be detected in untreated patients by widely available assays that measure copies of virion-associated HIV RNA in plasma (copies per milliliter). Levels of virus vary greatly in most untreated patients, usually ranging from several thousand to a few million copies of HIV RNA per milliliter of plasma. Studies using highly sensitive molecular techniques have demonstrated that even in certain patients in whom plasma viremia is suppressed to below detection (lower limit, 20–50 copies of HIV RNA per milliliter depending on assay kit manufacturer) by cART, there is a continual low level of virion production in the majority of infected patients. In other human viral infections, with very few exceptions, if the host survives, the virus is completely cleared from the body and a state of immunity against subsequent infection develops. HIV infection very rarely kills the host during primary infection. Certain viruses, such as HSV (Chap. 187), are

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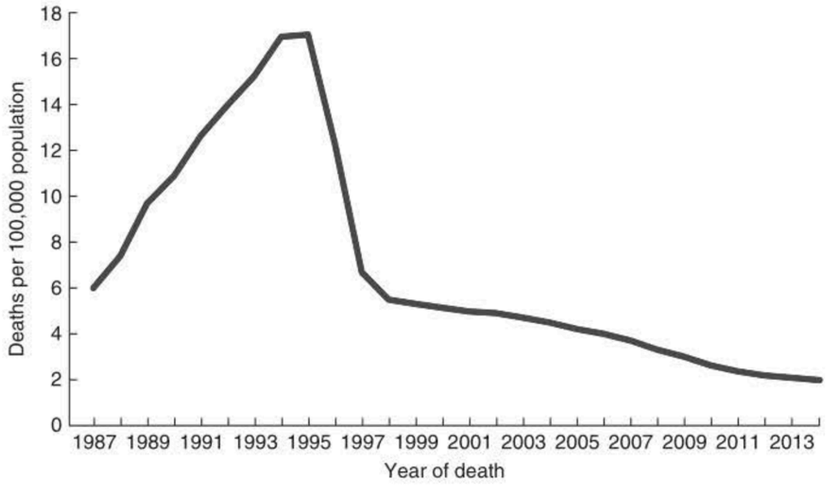

FIGURE 197-16 Trends in annual age-adjusted rates of death due to HIV infection, United States, 1987–2014. Age distribution based on 2000 population. *(From CDC.)*

not completely cleared from the body after infection, but instead enter a latent state; in these cases, clinical latency is accompanied by microbiologic latency. This is not the case with HIV infection as described above. Chronicity associated with persistent virus replication can also be seen in certain cases of HBV and HCV infections (**Chap. 334**); however, in these infections the immune system is not a target of the virus.

Escape of HIV from Effective Immune System Control

Inherent to the establishment of chronicity of HIV infection is the ability of the virus to evade adequate control and elimination by both the cellular and humoral immune responses. There are a number of mechanisms whereby the virus accomplishes this evasion. Paramount among these is the establishment of a sustained level of replication associated with the generation of viral diversity via mutation and recombination. The selection of mutants that escape control by CD8+ cytolytic T lymphocytes (CTLs) is critical to the propagation and progression of HIV infection. The high rate of virus replication associated with inevitable mutations also contributes to the inability of antibody to clear the autologous virus. Furthermore, for reasons that remain unclear, the humoral immune system does not readily produce classic neutralizing antibodies against the HIV envelope and does so only after years of persistent virus replication and after the infection is firmly established (see below). Extensive analyses of sequential HIV isolates and host responses have demonstrated that viral escape from B cell and CD8+ T cell responses occurs early after infection and allows the virus to stay one step ahead of effective immune responses. Virus-specific CD8+ CTLs expand greatly during primary HIV infection, and they likely represent the high-affinity responses that would be expected to be most efficient in eliminating virus-infected cells; however, viral control is generally incomplete as viral replication persists at relatively high levels in the majority of individuals. In addition to viral escape from CTLs through high rates of mutation, it is thought that the initially strong response becomes qualitatively dysfunctional owing to the overwhelming immune activation associated with persistent viral replication, leading to immune "exhaustion" that affects both arms of adaptive immunity. Several studies have indicated that exhaustion of HIV-specific CD8+ T cells during prolonged immune activation is associated with upregulation of several inhibitory receptors, such as programmed death (PD) 1 molecule (of the B7-CD28 family of molecules), T cell immunoreceptor with Ig and ITIM domains (TIGIT), T cell immunoglobulin and mucin-domain containing molecule 3 (Tim-3), and lymphocyte activating gene 3 (Lag-3), collectively referred to as *immune-checkpoint receptors*. Upregulation of these surface proteins restricts polyreactivity and proliferative capacity, functional attributes of CD8+ T cells that are essential for effective killing of pathogens. Another mechanism contributing to the evasion by HIV of immune system control is the downregulation of HLA class I molecules on the surface of HIV-infected cells by the viral proteins Nef, Tat, and Vpu, resulting in the lack of ability of CD8+ CTLs to recognize and kill infected target cells. Although this downregulation of HLA class I molecules would seem to favor elimination of HIV-infected cells by natural killer (NK) cells, this latter mechanism does not remove HIV-infected cells effectively (see below). Another potential means of escape of HIV-infected cells from elimination by CD8+ CTLs is the sequestration of infected cells in immunologically privileged sites such as the central nervous system (CNS), as well as the low frequency of virus-specific

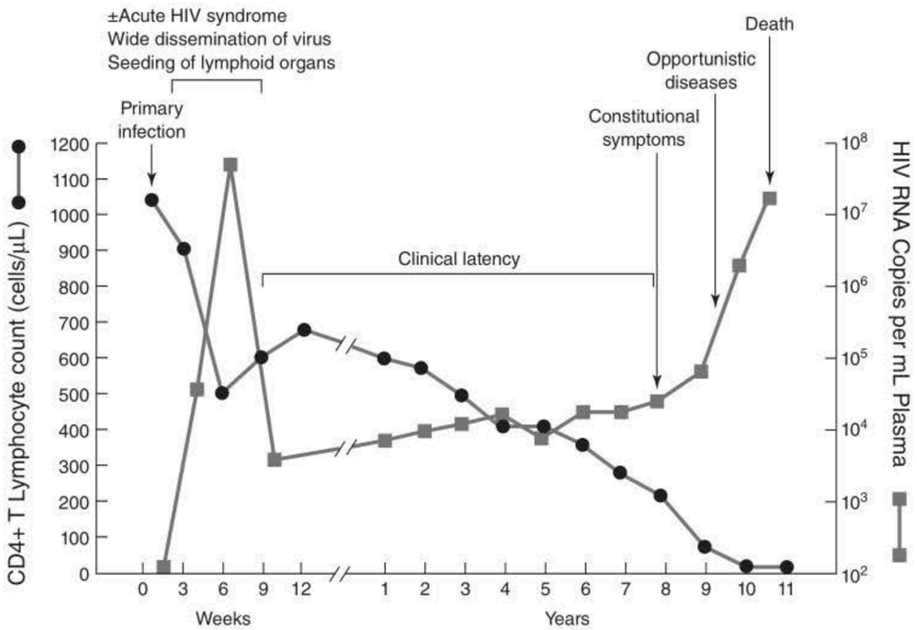

FIGURE 197-17 Typical course of an untreated HIV-infected individual. See text for detailed description. *(From G Pantaleo et al: N Engl J Med 328:327, 1993. Copyright 1993 Massachusetts Medical Society. All rights reserved.)*

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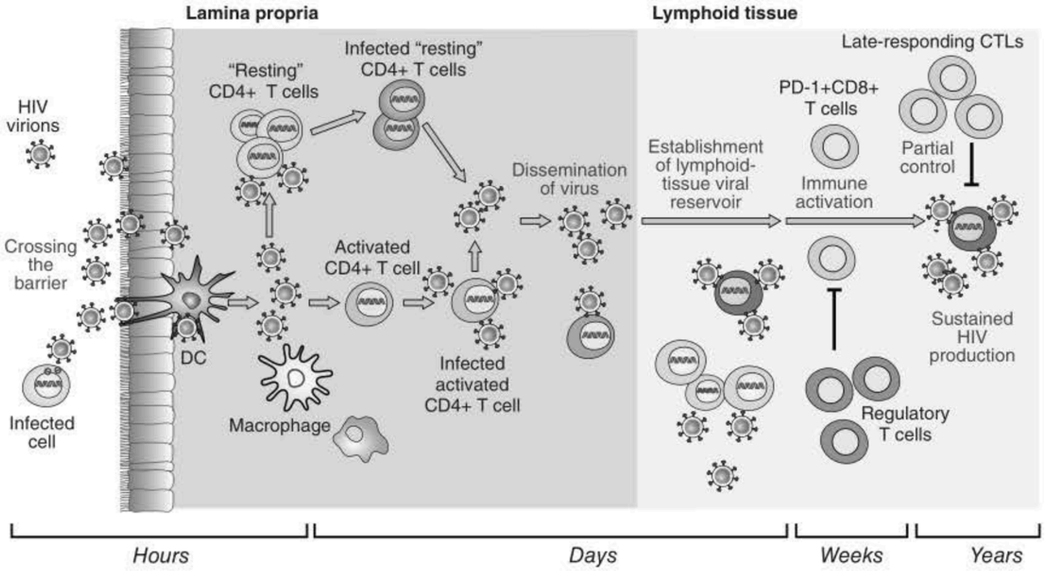

FIGURE 197-18 Summary of early events in HIV infection. See text for detailed description. CTLs, cytolytic T lymphocytes; HIV, human immunodeficiency virus. *(Adapted from AT Haase: Nat Rev Immunol 5:783, 2005.)*

CD8+ CTLs in areas of lymphoid tissues, namely germinal centers, where HIV actively replicates.

The principal targets of neutralizing antibodies against HIV are the envelope proteins gp120 and gp41. HIV employs at least three mechanisms to evade neutralizing antibody responses: hypervariability in the primary sequence of the envelope, extensive glycosylation of the envelope, and conformational masking of neutralizing epitopes. Several studies that have followed the evolution of the humoral immune response to HIV from the earliest points after primary infection indicate that the virus continually mutates to escape the emerging antibody response such that the sequential antibodies that are induced do not neutralize the currently autologous virus. *Broadly neutralizing antibodies* capable of neutralizing a wide range of primary HIV isolates in vitro occur in only about 20% of HIV-infected individuals, and, when they do occur, 2 to 3 years of infection with continual virus replication are generally required to drive the affinity maturation of the antibodies. Unfortunately, by the time these broadly neutralizing antibodies are formed, they are ineffective in containing the virus currently replicating in the patient. Persistent viremia also results in exhaustion of B cells similar to the exhaustion reported for CD8+ T cells, adding to the defects in the humoral response to HIV.

CD4+ T cell help is essential for the integrity of antigen-specific immune responses, both humoral and cell-mediated. HIV preferentially infects activated CD4+ T cells including HIV-specific CD4+ T cells, and so this loss of viral-specific helper T cell responses has profoundly negative consequences for the immunologic control of HIV replication. Furthermore, this loss occurs early in the course of infection, and animal studies indicate that 40–70% of all memory CD4+ T cells in the GALT are eliminated during acute infection. During chronic HIV viremia, CD4+ T cells also exhibit evidence of exhaustion, including by upregulation of the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), also a member of the B7-CD28 family.

Finally, the escape of HIV from immune-mediated elimination during primary infection allows the formation of a pool of latently infected cells, referred to as the *viral reservoir*, that may not be recognized or completely eliminated by virus-specific CTLs or by ART (see below). Thus, despite a potent immune response and the marked downregulation of virus replication following primary HIV infection, HIV succeeds in establishing a state of chronic infection with a variable degree of persistent virus replication. During this period most patients make the clinical transition from acute primary infection to variable periods of clinical latency or smoldering disease activity (see below).

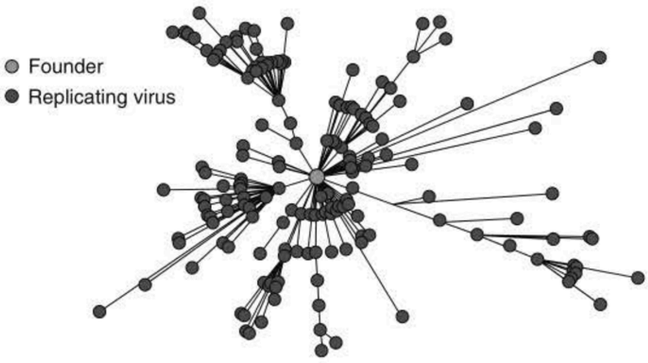

FIGURE 197-19 As HIV diverges from founder to chronically replicating virus, it accumulates N-linked glycosylation sites. See text for detailed description. *(Adapted from CA Derdeyn et al: Science 303:2019, 2004; B Chohan et al: J Virol 79:6528, 2005; and BF Keele et al: Proc Natl Acad Sci USA 105:7552, 2008.)*

The HIV Reservoir: Obstacles to the Eradication of Virus A pool of latently infected, resting CD4+ T cells that serves as at least one component of the persistent reservoir of virus exists in virtually all HIV-infected individuals, including those who are receiving cART. Such cells carry an integrated form of HIV DNA in the genome of the host and can remain in this state until an activation signal drives the expression of HIV transcripts. Only a small fraction of the latently infected cells in the viral reservoir contain replication-competent virus with the overwhelming majority of cells containing defective proviruses incapable of a full replication cycle. However, upon activation of the reservoir variable degrees of sustained virus replication invariably occur. This form of latency is to be distinguished from preintegration latency, in which HIV enters a resting CD4+ T cell and, in the absence of an activation signal, reverse transcription of the HIV genome occurs to a certain extent but the resulting proviral DNA fails to integrate into the host genome. This period of preintegration latency may last hours to days, and if no activation signal is delivered to the cell, the proviral DNA loses its capacity to initiate a productive infection. If these cells do become activated prior to decay of the preintegration complex, reverse transcription proceeds to completion and the virus continues along its replication cycle (see above and Fig. 197-20). The pool of cells that are in the postintegration state of latency is established early during the course of primary HIV infection. Despite the suppression of plasma viremia to undetectable levels by potent regimens of cART administered over several years, this pool of latently infected cells persists and can give rise to replication-competent virus upon cellular activation ex

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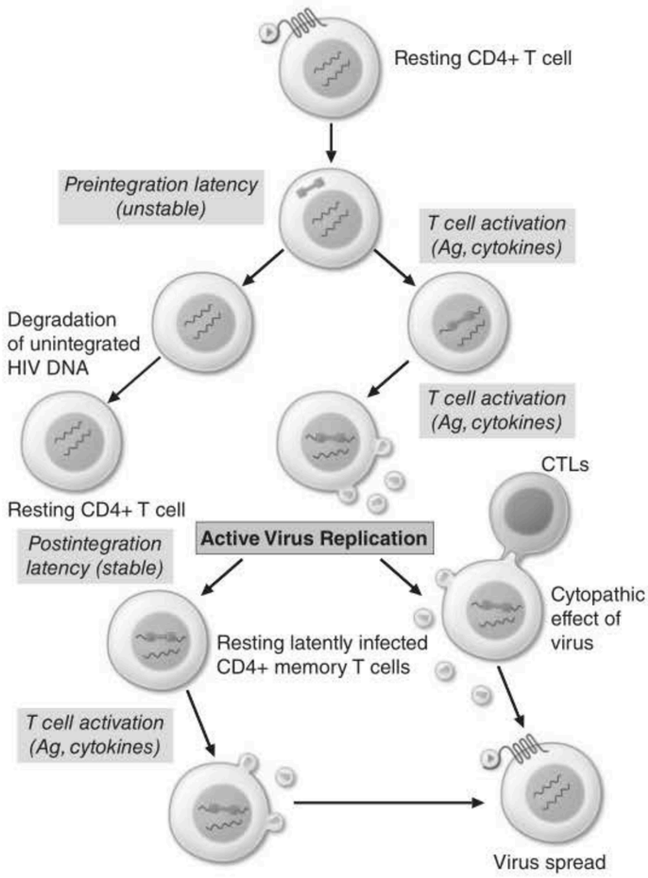

FIGURE 197-20 Generation of latently infected, resting CD4+ T cells in HIV-infected individuals. See text for details. Ag, antigen; CTLs, cytolytic T lymphocytes. *(Courtesy of TW Chun; with permission.)*

vivo. Modeling studies built on projections of decay curves have estimated that in such a setting of prolonged viral suppression, it would require a few to several years for the pool of latently infected cells to be completely eliminated. This has not been documented to occur spontaneously in any patients very likely because the latent viral reservoir is long-lived and is continually replenished by the low levels of persistent virus replication that may remain below the limits of detection of current assays (see below) as well as by the expansion by proliferation of the pool of latently infected cells (Fig. 197-20), even in patients who for the most part are treated successfully. Reservoirs of HIV-infected cells, latent or otherwise, can exist in a number of compartments including the lymphoid tissue, peripheral blood, and the CNS (likely in cells of the monocyte/macrophage lineage) as well as in other unidentified locations. Over the past several years attempts have been made to eliminate HIV in the latent viral reservoir using agents that activate resting CD4+ T cells during the course of cART; however, such attempts, referred to as "shock and kill," have been unsuccessful. Thus, this persistent reservoir of infected cells and/or low levels of persistent virus replication remain major obstacles to the goal of eradication of virus from infected individuals and hence a "cure," despite the favorable clinical outcomes that have resulted from cART.

Viral Dynamics The dynamics of viral production and turnover have been quantified using mathematical modeling in the setting of the administration of reverse transcriptase and protease inhibitors to HIV-infected individuals in clinical studies. Treatment with these drugs resulted in a precipitous decline in the level of plasma viremia, which typically fell by well over 90% within 2 weeks. It was determined on the basis of modeling the kinetics of viral decline and the emergence of resistant mutants during therapy that 93–99% of the circulating virus originated from recently infected, rapidly turning over CD4+ T cells and that ~1–7% of circulating virus originated from longer-lived cells, likely monocytes/macrophages. A negligible amount of circulating virus originated from the pool of latently infected cells (Fig. 197-21). It was also determined that the half-life of a circulating virion was ~30–60 min and that of productively infected cells was 1 day. Given the relatively steady level of plasma viremia and of infected cells, it appears that extremely large amounts of virus (~10^{10}–10^{11} virions) are produced and cleared from the circulation each day. In addition, data suggest that the minimal duration of the HIV-1 replication cycle in vivo is ~2 days. Other studies have demonstrated that the decrease in plasma viremia that results from cART correlates closely with a decrease in virus replication in lymph nodes, further confirming that lymphoid tissue is the main site of HIV replication and the main source of plasma viremia.

The level of steady-state viremia, called the viral *set point*, at ~1 year following acquisition of HIV infection has important prognostic implications for the progression of HIV disease in the untreated patient. It has been demonstrated that, as a group, untreated HIV-infected individuals who have a low set point at 6 months to 1 year following infection progress to AIDS much more slowly than do individuals whose set point is very high at that time (Fig. 197-22).

Clinical Latency versus Microbiologic Latency With the exception of certain long-term nonprogressors and "elite controllers" of HIV replication, the level of CD4+ T cells in the blood inevitably decreases progressively in viremic HIV-infected individuals in the absence of cART. The decline in CD4+ T cells may be gradual or abrupt, the latter usually reflecting a significant spike in the level of plasma viremia. Most patients are relatively asymptomatic while this progressive decline is taking place (see below) and are often described as being in a state of *clinical latency*. However, this term is misleading; it does not mean disease latency, since progression, although slow in many cases and often without symptoms, is generally relentless during this period. Furthermore, clinical latency should not be confused with microbiologic latency, since varying levels of virus replication inevitably occur during this period of clinical latency. Even in those rare patients who have <50 copies of HIV RNA per milliliter in the absence of therapy, there is virtually always some degree of low-level ongoing virus replication.

ADVANCED HIV DISEASE

In untreated patients or in patients in whom therapy has not adequately controlled virus replication, after a variable period, usually measured in years, the CD4+ T cell count falls below a critical level (<200/μL) and the patient becomes highly susceptible to opportunistic

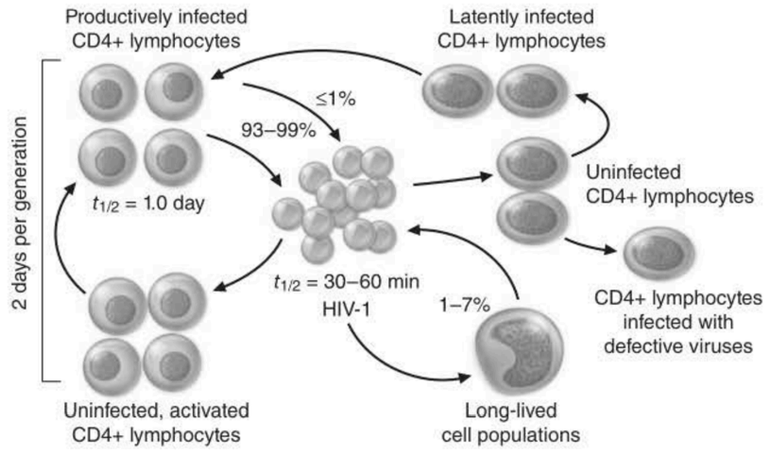

FIGURE 197-21 Dynamics of HIV infection in vivo. See text for detailed description. *(From AS Perelson et al: Science 271:1582, 1996.)*

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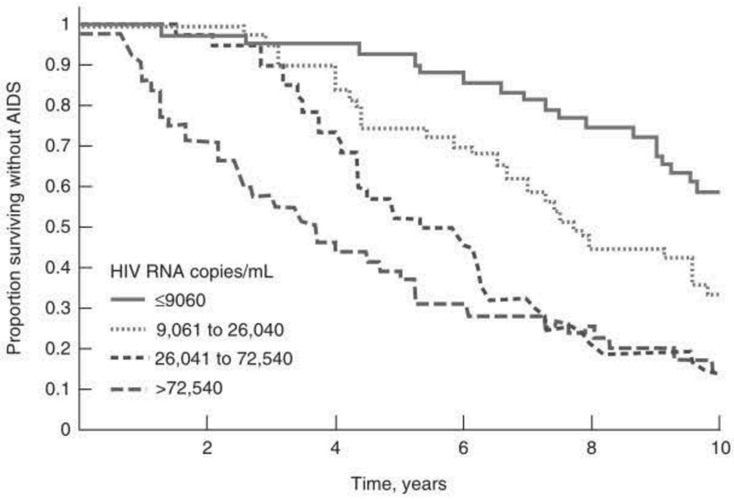

FIGURE 197-22 Relationship between levels of virus and rates of disease progression. Kaplan-Meier curves for AIDS-free survival stratified by baseline HIV-1 RNA categories (copies per milliliter). *(From JW Mellors et al: Science 272:1167, 1996.)*

disease (Fig. 197-17). For this reason, the CDC case definition of AIDS includes all HIV-infected individuals >5 years of age with CD4+ T cell counts below this level (Table 197-2). Patients may experience constitutional signs and symptoms or may develop an opportunistic disease abruptly without any prior symptoms. The depletion of CD4+ T cells continues to be progressive and unrelenting in this phase. It is not uncommon for CD4+ T cell counts in the untreated patient to drop to as low as 10/μL or even to zero. In countries where cART and prophylaxis and treatment for opportunistic infections are readily accessible to such patients, survival is increased dramatically even in those patients with advanced HIV disease. In contrast, untreated patients who progress to this severest form of immunodeficiency usually succumb to opportunistic infections or neoplasms (see below).

LONG-TERM SURVIVORS, LONG-TERM NONPROGRESSORS, AND ELITE CONTROLLERS

It is important to distinguish between the terms *long-term survivor* and *long-term nonprogressor*. Long-term nonprogressors are by definition long-term survivors; however, the reverse is not always true. Predictions from one study that antedated the availability of effective cART estimated that ~13% of homosexual/bisexual men who were infected at an early age may remain free of clinical AIDS for >20 years. Many of these individuals may have progressed in their degree of immune deficiency; however, they certainly survived for a considerable period of time. With the advent of effective cART, the survival of HIV-infected individuals has dramatically increased. Early in the AIDS epidemic, prior to the availability of therapy, if a patient presented with a life-threatening opportunistic infection, the median survival was 26 weeks from the time of presentation. Currently, an HIV-infected 20-year-old individual in a high-income country who is appropriately treated with cART can expect to live at least 50 years according to mathematical model projections. In the face of cART, long-term survival is becoming commonplace. Definitions of long-term nonprogressors have varied considerably over the years, and so such individuals constitute a heterogeneous group. Long-term nonprogressors were first described in the 1990s. Originally, individuals were considered to be long-term nonprogressors if they had been infected with HIV for a long period (≥10 years), their CD4+ T cell counts were in the normal range, and they remained stable over years without receiving cART. Approximately 5–15% of HIV-infected individuals fell into this broader nonprogressor category. However, this group was rather heterogenous and over time a significant proportion of these individuals progressed and ultimately required therapy. From this broader group, a much smaller subgroup of "elite" controllers or nonprogressors was identified, and they constituted a fraction of 1% of HIV-infected individuals. These elite controllers, by definition, have extremely low levels of plasma viremia that is often undetectable by standard assays and normal CD4+ T cell counts. It is noteworthy that certain of their HIV-specific immune responses are robust and clearly superior to those of HIV-infected progressors. In this group of elite controllers certain HLA class I haplotypes are overrepresented, particularly HLA-B57-01 and HLA-B27-05. Outside of the subgroup of elite controllers, a number of other genetic factors have been shown to be involved to a greater or lesser degree in the control of virus replication and thus in the rate of HIV disease progression (see "Genetic Factors in HIV-1 and AIDS Pathogenesis," below).

LYMPHOID ORGANS AND HIV PATHOGENESIS

Regardless of the portal of entry of HIV, lymphoid tissues are the major anatomic sites for the establishment and propagation of HIV infection. Despite the use of measurements of plasma viremia to determine the level of disease activity, virus replication occurs mainly in lymphoid tissue and not in blood; indeed, the level of plasma viremia directly reflects virus production in lymphoid tissue.

Some patients experience progressive generalized lymphadenopathy early in the course of the infection; others experience varying degrees of transient lymphadenopathy. Lymphadenopathy reflects the cellular activation and immune response to the virus in the lymphoid tissue, which is generally characterized by follicular or germinal center hyperplasia. Lymphoid tissue involvement is a common denominator of virtually all patients with HIV infection, even those without easily detectable lymphadenopathy.

Examinations of lymph tissue and peripheral blood in patients and monkeys during various stages of HIV and SIV infection, respectively, have led to substantial insight into the pathogenesis of HIV disease. In most of the original human studies, peripheral lymph nodes have been used predominantly as the source of lymphoid tissue. More recent studies in monkeys and humans have also focused on the GALT, where the earliest burst of virus replication occurs associated with marked depletion of CD4+ T cells. In detailed studies of peripheral lymph node tissue that utilized a variety of molecular techniques to measure the level of HIV DNA and RNA and imaging techniques to visualize virus and cells, the following picture has emerged. During acute HIV infection resulting from mucosal transmission, virus replication progressively amplifies from scattered lymphoid cells in the lamina propria of the gut to draining lymphoid tissue, leading to high levels of plasma viremia. The GALT plays a major role in the amplification of virus replication, and virus is disseminated from replication in the GALT to peripheral lymphoid tissue. A profound degree of cellular activation occurs within lymphoid tissue (see below) and is reflected in follicular or germinal center hyperplasia. At this time copious amounts of extracellular virions (both infectious and defective) are trapped on the processes of the follicular dendritic cells (FDCs) in the germinal centers of the lymph nodes. Virions that have bound complement components on their surfaces attach to the surface of FDCs via interactions with complement receptors and likely via Fc receptors that bind to antibodies that are attached to the virions. In situ hybridization reveals expression of virus in individual cells of the paracortical area and, to a lesser extent, the germinal center (Fig. 197-23). The persistence of trapped virus likely reflects a steady state whereby trapped virus turns over and is replaced by fresh virions that are continually produced. The trapped virus, either as whole virion or shed envelope, serves as a continual activator of CD4+ T cells, thus driving further virus replication.

During the early stages of HIV disease, the architecture of lymphoid tissues is generally preserved and may even be hyperplastic owing to an increased presence of B cells and specialized CD4+ T cells called follicular helper CD4+ T cells (TF_H) in prominent germinal centers. Extracellular virions can be seen by electron microscopy attached to FDC processes. The trapping of antigen is a physiologically normal function for the FDCs, which present antigen to B cells and, along with stimulatory factors produced by TF_H cells, contribute to the generation of B cell memory. However, in the case of HIV, persistent cellular

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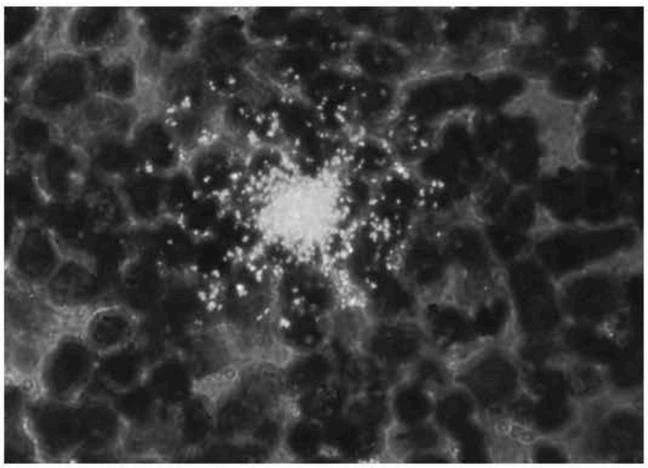

FIGURE 197-23 HIV in the lymph node of an HIV-infected individual. An individual cell infected with HIV shown expressing HIV RNA by in situ hybridization using a radiolabeled molecular probe. Original ×500. *(Adapted from G Pantaleo, AS Fauci et al: Nature 362:355, 1993.)*

activation, resulting in a shift to secretion of proinflammatory cytokines such as interleukin (IL) 1β, tumor necrosis factor (TNF) α, IFN-γ, and IL-6, can induce viral replication (see below) and diminish the effectiveness of the immune response against the virus. In addition, the CD4+ TF_H cells that are recruited into the germinal center to provide help to B cells in the generation of an HIV-specific immune response are highly susceptible to infection and may be an important component of the HIV reservoir. Thus, in HIV infection, a normal physiologic function of the immune system that contributes to the clearance of virus, as well as to the generation of a specific immune response, can also have deleterious consequences.

As HIV disease progresses, the architecture of lymphoid tissues begins to show disruption. Confocal microscopy reveals destruction of the fibroblastic reticular cell (FRC) and FDC networks in the T cell zone and B cell follicles, respectively. The mechanisms of destruction are not completely understood, but they are thought to be associated with collagen deposition causing fibrosis and loss of production of cytokines such as IL-7 and lymphotoxin-α, which are critical to the maintenance of lymphoid tissues and their lymphocyte constituents. As the disease progresses to an advanced stage, there is complete disruption of the architecture of the lymphoid tissues, accompanied by dissolution of the FRC and FDC networks. At this point, the lymph nodes are "burnt out." This destruction of lymphoid tissue compounds the immunodeficiency of HIV disease and contributes both to the inability to control HIV replication and to the inability to mount adequate immune responses against opportunistic pathogens. The events from primary infection to the ultimate destruction of the immune system are illustrated in **Fig. 197-24**. More recently, nonhuman primate studies and some human studies have examined GALT at various stages of HIV disease. Within the GALT, the basal level of cellular activation combined with virus-mediated activation results in the infection and elimination of an estimated 50–90% of CD4+ T cells in the gut. The extent of this early damage to GALT, which constitutes a major component of lymphoid tissue in the body, may play a role in determining the potential for immunologic recovery of the memory cell subset.

THE ROLE OF IMMUNE ACTIVATION AND INFLAMMATION IN HIV PATHOGENESIS

Activation of the immune system and variable degrees of inflammation are essential components of any appropriate immune response to a foreign antigen. However, immune activation and inflammation, which can be considered aberrant in HIV-infected individuals, play a critical role in the pathogenesis of HIV disease as well as other chronic conditions associated with HIV infection. Immune activation and inflammation in the HIV-infected individual contribute substantially to (1) the replication of HIV, (2) the induction of immune dysfunction, and (3) the increased incidence of chronic conditions such as premature cardiovascular disease **(Table 197-4)**.

INDUCTION OF HIV REPLICATION BY ABERRANT IMMUNE ACTIVATION The immune system is normally in a state of homeostasis, awaiting perturbation by foreign antigenic stimuli. Once the immune response deals with and clears the antigen, the system returns to relative quiescence **(Chap. 342)**. This is generally not the case in HIV infection where, in the untreated patient, virus replication is invariably persistent with very few exceptions and immune activation is persistent. HIV replicates most efficiently in activated CD4+ T cells; in HIV infection, chronic activation provides the cell substrates necessary for persistent virus replication throughout the course of HIV disease, particularly in the untreated patient. Even in certain patients receiving cART whose levels of plasma viremia are suppressed to below the level of detection by standard assays, there are low but detectable degrees of virus replication that drives persistent immune activation. From a virologic standpoint, although quiescent CD4+ T cells can be infected, albeit inefficiently, with HIV, reverse transcription, integration, and virus spread are much more efficient in activated cells. Furthermore, cellular activation induces expression of virus in cells latently infected with HIV. In essence, immune activation and inflammation provide the engine that drives HIV replication. In addition to endogenous factors such as cytokines, a number of exogenous factors such as other microbes that are associated with heightened cellular activation can enhance HIV replication and thus may play a role in HIV pathogenesis. Co-infection with a range of viruses, such as HSV types 1 and 2, cytomegalovirus (CMV), human herpesvirus (HHV) 6, Epstein-Barr virus (EBV), HBV, HCV, adenovirus, and HTLV-1 have been shown to upregulate HIV expression. In addition, infestation with nematodes has been shown to be associated with a heightened state of immune activation that facilitates HIV replication; in certain studies deworming of the infected host has resulted in a decrease in plasma viremia.

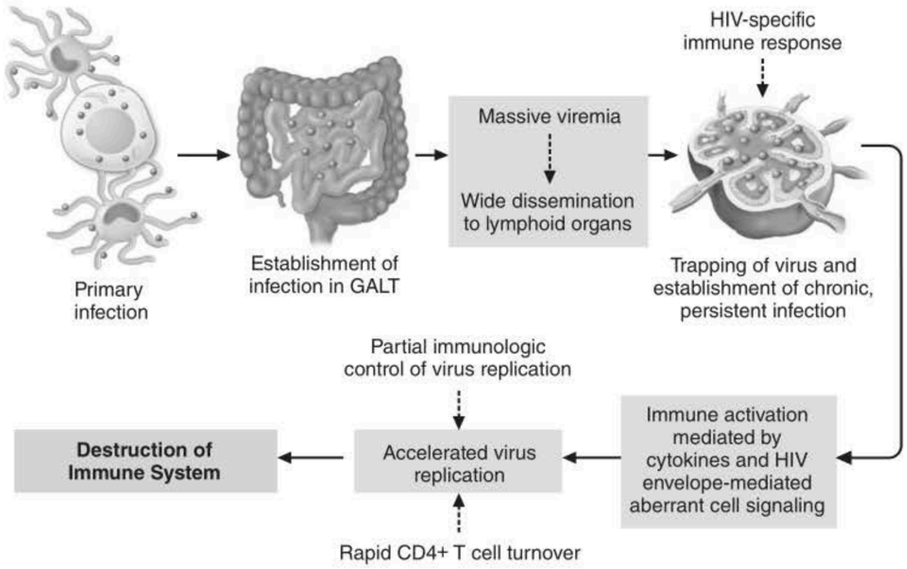

FIGURE 197-24 Events that transpire from primary HIV infection through the establishment of chronic persistent infection to the ultimate destruction of the immune system. See text for details. CTLs, cytolytic T lymphocytes; GALT, gut-associated lymphoid tissue.

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TABLE 197-4 Conditions Associated with Persistent Immune Activation and Inflammation in Patients with HIV Infection

Accelerated aging syndrome
Bone fragility
Cancers
Cardiovascular disease
Diabetes
Kidney disease
Liver disease
Neurocognitive dysfunction

Two diseases of extraordinary global health significance, malaria and tuberculosis (TB), have been shown to increase HIV viral load in dually infected individuals. Globally, *Mycobacterium tuberculosis* is the most common opportunistic infection in HIV-infected individuals (**Chap. 173**). In addition to the fact that HIV-infected individuals are more likely to develop active TB after exposure and to reactivate latent TB, it has been demonstrated that active TB can accelerate the course of HIV infection. It has also been shown that levels of plasma viremia are greatly elevated in HIV-infected individuals with active TB who are not receiving cART, compared with pre-TB levels and levels of viremia after successful treatment of the active TB. The situation is similar in the interaction between HIV and malaria parasites (**Chap. 219**). Acute infection of HIV-infected individuals with *Plasmodium falciparum* increases HIV viral load, and the increased viral load is reversed by effective treatment of malaria.

MICROBIAL TRANSLOCATION AND PERSISTENT IMMUNE ACTIVATION One proposed mechanism of persistent immune activation involves the disruption of the mucosal barrier in the gut due to HIV replication in submucosal lymphoid tissue. As a result of this disruption, there is an increase in the products, particularly lipopolysaccharide (LPS), of bacteria that translocate from the bowel lumen through the damaged mucosa to the circulation, leading to persistent systemic immune activation and inflammation. This effect can persist even after the HIV viral load is brought to <50 copies/mL by cART. Depletion in the GALT of IL-17–producing T cells, which are responsible for defense against extracellular bacteria and fungi, also is thought to contribute to HIV pathogenesis.

PERSISTENT IMMUNE ACTIVATION AND INFLAMMATION INDUCE IMMUNE DYSFUNCTION The immune activated state in HIV infection is reflected by hyperactivation of B cells leading to hypergammaglobulinemia; increased lymphocyte turnover; activation of monocytes; expression of activation markers and immune checkpoint receptors on CD4+ and CD8+ T cells; increased activation-associated cellular apoptosis; lymph node hyperplasia, particularly early in the course of disease; increased secretion of proinflammatory cytokines, particularly IL-6; elevated levels of high-sensitivity C-reactive protein, fibrinogen, D-dimer, neopterin, β_2-microglobulin, acid-labile interferon, soluble (s) IL-2 receptors (R), sTNFR, sCD27, and sCD40L; and autoimmune phenomena (see "Autoimmune Phenomena," below). Even in the absence of direct infection of a target cell, HIV envelope proteins can interact with cellular receptors (CD4 molecules and chemokine receptors) to deliver potent activation signals resulting in calcium flux, the phosphorylation of certain proteins involved in signal transduction, co-localization of cytoplasmic proteins including those involved in cell trafficking, immune dysfunction, and, under certain circumstances, apoptosis. From an immunologic standpoint, chronic exposure of the immune system to a particular antigen over an extended period of time may ultimately lead to an inability to sustain an adequate immune response to the antigen in question. In many chronic viral infections, including HIV infection, persistent viremia is associated with "functional exhaustion" of virus-specific T cells, decreasing their capacity to proliferate and perform effector functions. It has been demonstrated that this phenomenon of immune exhaustion may be mediated, at least in part, by the upregulation of inhibitory receptors on HIV-specific T cells, such as PD-1 and Tim-3 that are shared by both CD4+ and CD8+ T cells, as well as CTLA-4 on CD4+ and 2B4 and CD106 on CD8+ T cells. Furthermore, the ability of the immune system to respond to a broad spectrum of non-HIV antigens may be compromised if immunocompetent bystander cells are maintained in a state of chronic activation.

The deleterious effects of chronic immune activation on the progression of HIV disease are well established. As in most conditions of persistent antigen exposure, the host must maintain sufficient activation of antigen (HIV)-specific responses but must also prevent excessive activation and potential immune-mediated damage to tissues. Certain studies suggest that normal immunoregulatory mechanisms that act to keep hyperimmune activation in check, particularly CD4+, FoxP3+, and CD25+ regulatory T cells (T-regs), may be dysfunctional or depleted in the context of advanced HIV disease.

Apoptosis Apoptosis is a form of programmed cell death that is a normal mechanism for the elimination of effete cells in organogenesis as well as in the cellular proliferation that occurs during a normal immune response (**Chap. 342**). Apoptosis can occur by intrinsic or extrinsic pathways, the latter of which is largely dependent on cellular activation, and the aberrant cellular activation associated with HIV disease is correlated with a heightened state of apoptosis. HIV can trigger activation-induced cell death through the upregulation of the death receptors, such as Fas/CD95, TNFR1, or TNF-related apoptosis-inducing ligand (TRAIL) receptors 1 and 2. Their corresponding ligands FasL, TNF, and TRAIL also are upregulated in HIV disease. HIV-induced stress and alterations in homeostasis also can trigger intrinsic apoptosis due to the downregulation of antiapoptotic proteins such as Bcl-2. Other mechanisms of HIV-induced cell death have been described, including autophagy, necrosis, necroptosis, and pyroptosis. The phenomenon of pyroptosis, an inflammatory form of cell death involving the upregulation of the proinflammatory enzyme caspase 1 and release of the proinflammatory cytokines IL-1β and IL-18, has been linked to a bystander effect of HIV replication on CD4+ T cells. The process of pyroptosis generates multimeric complexes called inflammasomes, which can also be activated by LPS. Certain viral gene products have been associated with enhanced susceptibility to apoptosis; these include Env, Tat, and Vpr. In contrast, Nef has been shown to possess antiapoptotic properties. The intensity of apoptosis correlates with the general state of activation of the immune system and not with the stage of disease or with viral burden. A number of studies, including those examining lymphoid tissue, have demonstrated that the rate of apoptosis is elevated in HIV infection and that apoptosis is seen in "bystander" cells such as CD8+ T cells and B cells as well as in uninfected CD4+ T cells. It is likely that this bystander apoptosis of immunocompetent cells related to immune activation contributes to the general immune abnormalities in HIV disease.

MEDICAL CONDITIONS ASSOCIATED WITH PERSISTENT IMMUNE ACTIVATION AND INFLAMMATION IN HIV DISEASE It has become clear, as the survival of HIV-infected individuals has increased, that a number of previously unrecognized medical complications are associated with HIV disease—and that these complications relate to chronic immune activation and inflammation (Table 197-4). These complications can appear even after patients have experienced years of adequate control of viral replication (plasma viremia below detectable levels) for several years. Of particular note are endothelial cell dysfunction and its relationship to cardiovascular disease. Other chronic conditions that have been reported include bone fragility, certain cancers, diabetes, kidney and liver disease, and neurocognitive dysfunction, thus presenting an overall picture of accelerated aging.

Autoimmune Phenomena Autoimmune phenomena are commonly observed in HIV-infected individuals and they reflect, at least in part, chronic immune activation and the dysregulation of B and T cells. Although these phenomena usually occur in the absence of autoimmune disease, a wide spectrum of clinical manifestations that may be associated with autoimmunity have been described (see "Immunologic and Rheumatologic Diseases," below). Autoimmune phenomena include antibodies against autoantigens expressed on intact lymphocytes and other cells, or against proteins released from dying cells. Antiplatelet antibodies have some clinical relevance in that they may contribute to

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the thrombocytopenia of HIV disease (see below). Antibodies to nuclear and cytoplasmic components of cells have been reported, as have antibodies to cardiolipin and phospholipids; CD4 molecules; CD43 molecules; C1q-A; variable regions of the T cell receptor α, β, and γ chains; Fas; denatured collagen; and IL-2. In addition, autoantibodies to a range of serum proteins, including albumin, immunoglobulin, and thyroglobulin, have been reported. Molecular mimicry, either from opportunistic pathogens or from HIV itself, also is a trigger or cofactor in autoimmunity. Antibodies against the HIV envelope proteins, especially gp41, often cross-react with host proteins; the best known examples are antibodies directed against the membrane-proximal external region (MPER) of gp41 that also react with phospholipids and cardiolipin. The phenomenon of polyreactive HIV-specific antibodies may be beneficial to the host (see "Immune Response to HIV," below).

The increased occurrence and/or exacerbation of certain autoimmune diseases have been reported in HIV infection; these diseases include psoriasis, idiopathic thrombocytopenic purpura, Graves' disease, antiphospholipid syndrome, and primary biliary cirrhosis. The majority of these manifestations were described prior to the advent of cART and have decreased in frequency since its widespread use. However, with increasing availability of cART, an *immune reconstitution inflammatory syndrome* (IRIS) has been increasingly observed in infected individuals, particularly those with low CD4+ T cell counts (see below). IRIS is an autoimmune-like phenomenon characterized by a paradoxical deterioration of clinical condition, which is usually compartmentalized to a particular organ system, in individuals in whom cART has recently been initiated. It is associated with a decrease in viral load and at least partial recovery of immune competence, which is usually associated with increases in CD4+ T cell counts. The immunopathogenesis of this syndrome is felt to be related to an increase in immune response against the presence of residual antigens that are usually microbial and is most commonly seen with underlying *Mycobacterium tuberculosis* and cryptococcosis. This syndrome is discussed in more detail below.

CYTOKINES AND OTHER SOLUBLE FACTORS IN HIV PATHOGENESIS

The immune system is homeostatically regulated by a complex network of immunoregulatory cytokines, which are pleiotropic and redundant and operate in an autocrine and paracrine manner. They are expressed continuously, even during periods of apparent quiescence of the immune system. On perturbation of the immune system by antigenic challenge, the expression of cytokines increases to varying degrees (**Chap. 342**). Cytokines that are important components of this immunoregulatory network are thought to play major roles in HIV disease, during both the early and chronic phases of infection. A potent proinflammatory "cytokine storm" is induced during the acute phase of HIV infection, likely a response by inflammatory cells to virus replicating at very high levels. Cytokines that are induced during this early phase include IFN-α, IL-15, and the CXC chemokine IP-10 (CXCL10), followed by IL-6, IL-12, and TNF-α, and a delayed peak of the anti-inflammatory cytokine IL-10. Soluble factors of innate immunity also are induced shortly after infection, including neopterin and β-microglobulin. Several of these early-expressed cytokines and factors are not downregulated following the early phase of HIV infection, as seen in other self-resolving viral infections, and persist during the chronic phase of infection and contribute to maintaining high levels of immune activation. Among the cytokines and factors associated with early innate immune responses, they are intended to contain viral replication, although paradoxically most are potent inducers of HIV expression/replication because of their ability to induce immune activation that leads to enhanced viral production and an increase in readily available target cells for HIV (activated CD4+ T cells). The induction of IFN-α, one of the first cytokines induced during primary HIV infection and an important element of innate immune sensing, is thought to play a particularly important role in HIV pathogenesis by inducing a large number of IFN-associated genes that activate the immune system, alter the homeostasis of CD4+ T cells, and influence the virus variants that are selected during the HIV transmission bottleneck. Other cytokines that are elevated during the chronic phase of HIV infection and linked to immune activation include IFN-γ, the *CC-chemokine* RANTES (CCL5), macrophage inflammatory protein (MIP)-1β (CCL4), and IL-18.

Several specific cytokines and soluble factors have been associated with HIV pathogenesis at various stages of disease, in various tissues or organs, and in the regulation of HIV replication. Plasma levels of IP-10 are predictive of disease progression, whereas the proinflammatory cytokine IL-6, soluble CD14 (sCD14), and coagulation marker D-dimer are associated with increased risk of all-cause mortality in HIV-infected individuals. In particular, IL-6, sCD14, and D-dimer are associated with increased risk of cardiovascular disease and other causes of death, even in individuals receiving cART. IL-18 has also been shown to play a role in the development of the HIV-associated lipodystrophy syndrome, whereas increased levels of transforming growth factor (TGF)-β are associated with the induction of collagen deposition in lymph nodes (see above). Elevated levels of TNF-α and IL-6 have been demonstrated in plasma and cerebrospinal fluid (CSF), and increased expression of TNF-α, IL-1β, IFN-γ, and IL-6 has been demonstrated in the lymph nodes of HIV-infected individuals. RANTES, MIP-1α (CCL3), and MIP-1β (CCL4) (**Chap. 342**) inhibit infection by and spread of R5 HIV-1 strains, while *stromal cell–derived factor* (SDF) 1 inhibits infection by and spread of X4 strains. The mechanisms whereby the CC-chemokines RANTES (CCL5), MIP-1α (CCL3), and MIP-1β (CCL4) inhibit infection of R5 strains of HIV, or SDF-1 blocks X4 strains of HIV, involve blocking of the binding of the virus to its co-receptors, the CC-chemokine receptor CCR5 and the CXC-chemokine receptor CXCR4, respectively. Other soluble factors that have not yet been fully characterized, such as soluble CD8 antiviral factor (CAF), also have been shown to suppress HIV replication, independent of co-receptor usage.

LYMPHOCYTE TURNOVER IN HIV INFECTION

The immune systems of patients with HIV infection are characterized by a profound increase in lymphocyte turnover that is immediately reduced with effective cART. Studies utilizing in vivo or in vitro labeling of lymphocytes in the S-phase of the cell cycle have demonstrated a tight correlation between the degree of lymphocyte turnover and plasma levels of HIV RNA. This increase in turnover is seen in CD4+ and CD8+ T lymphocytes as well as B lymphocytes and can be observed in peripheral blood and lymphoid tissue. Mathematical models derived from these data suggest that one can view the lymphoid pool as consisting of dynamically distinct subpopulations of cells that are differentially affected by HIV infection. A major consequence of HIV infection appears to be a shift in cells from a more quiescent pool to a pool with a higher turnover rate. It is likely that a consequence of a higher rate of turnover is a higher rate of cell death. It has been suggested that the more rapid decline in CD4+ compared with CD8+ T cells may be linked to alterations in inflammatory and homeostatic cytokines that cause increased activation-induced death without replenishment of CD4+ but not CD8+ T cells. (See **Table 197-5** for additional mechanisms of depletion.)

THE ROLE OF VIRAL RECEPTORS AND CO-RECEPTORS IN HIV PATHOGENESIS

CCR5 AND CXCR4 As mentioned above, HIV-1 utilizes two major co-receptors along with CD4 to bind to, fuse with, and enter target cells; these co-receptors are CCR5 and CXCR4, which are also receptors for certain endogenous chemokines. Strains of HIV that utilize CCR5 as a co-receptor are referred to as *R5 viruses*. Strains of HIV that utilize CXCR4 are referred to as *X4 viruses*. Many virus strains are *dual tropic* in that they utilize both CCR5 and CXCR4; these are referred to as *R5X4 viruses*.

The natural chemokine ligands for the major HIV co-receptors can readily block entry of HIV. For example, the CC-chemokines RANTES (CCL5), MIP-1α (CCL3), and MIP-1β (CCL4), which are the natural ligands for CCR5, block entry of R5 viruses, whereas SDF-1, the natural ligand for CXCR4, blocks entry of X4 viruses. The mechanism of inhibition of viral entry is a steric inhibition of binding that is not dependent on signal transduction (**Fig. 197-25**).

The transmitting virus is almost invariably an R5 virus that predominates during the early stages of HIV disease. In the absence of cART

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TABLE 197-5 Proposed Mechanisms of CD4+ T Cell Dysfunction and Depletion

DIRECT MECHANISMS	INDIRECT MECHANISMS
Loss of plasma membrane integrity due to viral budding	Aberrant intracellular signaling events
Accumulation of unintegrated viral DNA Activation of DNA-dependent protein kinase during viral integration into host genome	Autoimmunity
Interference with cellular RNA processing	Innocent bystander killing of viral antigen–coated cells
Intracellular gp120-CD4 autofusion events	Apoptosis, pyroptosis (caspase-1 associated inflammation), autophagy
Syncytia formation	Inhibition of lymphopoiesis from reduced survival cytokines and lymphoid tissue integrity Activation-induced cell death Elimination of HIV-infected cells by virus-specific immune responses

or in cases of therapy failure, there is a transition to a predominantly X4 virus in approximately half of individuals infected with subtype B. The transition is often preceded by dual R5X4 strains, and detection of X4 variants is associated with a relatively rapid decline in CD4+ T cell counts, increased HIV plasma viremia, and progression of disease. However, the other half of infected individuals progress in their disease while maintaining predominance of an R5 virus, and individuals infected with subtype C rarely switch from CCR5 tropism to CXCR4 tropism. The reason for this difference is unclear.

The basis for the tropism of different envelope glycoproteins for either CCR5 or CXCR4 relates to the ability of the HIV envelope, including the third variable region (V3 loop) of gp120, to interact with these co-receptors. In this regard, binding of gp120 to CD4 induces a conformational change in gp120 that increases its affinity for the relevant co-receptor. Finally, R5 viruses are more efficient in infecting monocytes/macrophages and microglial cells of the brain (see "Neuropathogenesis in HIV Disease," below).

THE INTEGRIN α4β7 The integrin α4β7 is an accessory receptor for HIV. It is not essential for the binding and infection of a CD4+ T cell by HIV; however, it likely plays an important role in the transmission of HIV at mucosal surfaces such as the genital tract and gut and contributes somewhat to the pathogenesis of HIV disease. The integrin α4β7, which is the gut homing receptor for peripheral T cells, binds in its activated form to a specific tripeptide in the V2 loop of gp120, resulting in rapid activation of leukocyte function–associated antigen 1 (LFA-1), the central integrin in the establishment of virologic synapses, which facilitate efficient cell-to-cell spread of HIV. It has been demonstrated that $\alpha4\beta7^{high}$ CD4+ T cells are more susceptible to productive infection than are $\alpha4\beta7^{low-neg}$ CD4+ T cells because this cellular subset is enriched with metabolically active CD4+ T cells that are $CCR5^{high}$. These cells are present at the gut and genital tract mucosal surfaces. Importantly, it has been demonstrated that the virus that is transmitted during sexual exposure binds much more efficiently to α4β7 than does the virus that diversifies from the transmitting virus over time by mutation, particularly involving the accumulation of glycogens on the surface of the HIV envelope (see "Early Events in HIV Infection: Primary Infection and Initial Dissemination of Virus," above).

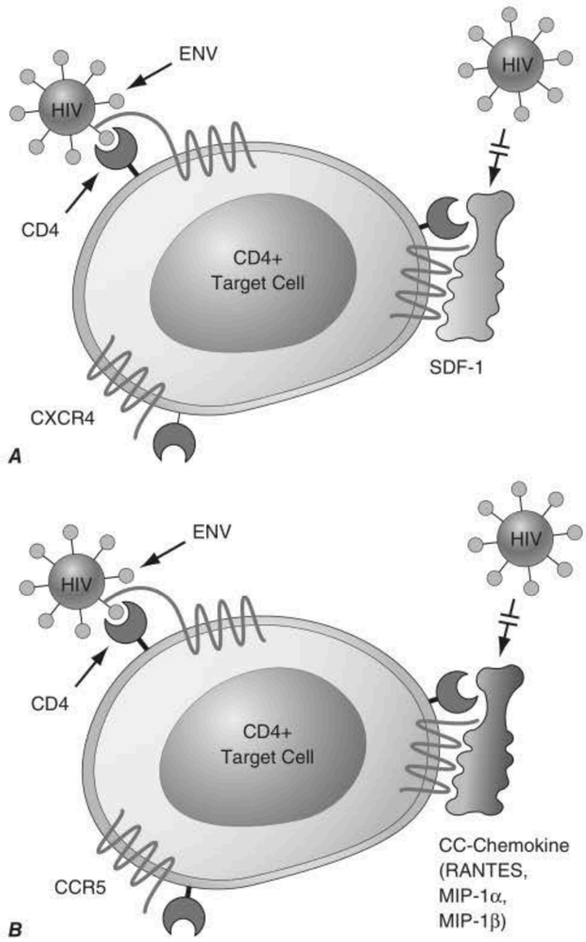

FIGURE 197-25 Model for the role of co-receptors CXCR4 and CCR5 in the efficient binding and entry of X4 (***A***) and R5 (***B***) strains of HIV-1, respectively, into CD4+ target cells. Blocking of this initial event in the virus life cycle can be accomplished by inhibition of binding to the co-receptor by the normal ligand for the receptor in question. The ligand for CXCR4 is stromal cell–derived factor (SDF-1); the ligands for CCR5 are RANTES, MIP-1α, and MIP-1β.

CELLULAR TARGETS OF HIV

CD4+ T lymphocytes and to a lesser extent CD4+ cells of the myeloid lineage are the principal targets of HIV and are the only cells that can be productively infected with HIV. Circulating DCs have been reported to express low levels of CD4, although high expression of the restriction factor SAMHD1 in myeloid (mDC) and plasmacytoid (pDC) DCs limits HIV replication in these cells by depleting intracellular pools of dNTPs and directly degrading viral RNA. Epidermal Langerhans cells express CD4 and have been infected by HIV in vivo, although, they too restrict replication by high expression of the host restriction factor, langerin. As has been shown in vivo for DCs, FDCs, and B cells, Langerhans cells are more likely to bind and transfer virus to activated CD4+ T cells than to be productively infected themselves.

Of potential clinical relevance is the demonstration that thymic precursor cells, which were assumed to be negative for CD3, CD4, and CD8 molecules, actually do express low levels of CD4 and can be infected with HIV in vitro. In addition, human thymic epithelial cells transplanted into an immunodeficient mouse can be infected with HIV by direct inoculation of virus into the thymus. Since these cells may play a role in the normal regeneration of CD4+ T cells, it is possible that their infection and depletion contribute, at least in part, to the impaired ability of the CD4+ T cell pool to completely reconstitute itself in certain infected individuals in whom cART has suppressed plasma viremia to below the level of detection (see below). In addition, CD34+ monocyte precursor cells have been shown to be infected in vivo in patients with advanced HIV disease. It is likely that these cells express low levels of CD4, and therefore it is not essential to invoke CD4-independent mechanisms to explain the infection. The clinical relevance of this finding is unclear.

QUALITATIVE AND QUANTITATIVE ABNORMALITIES OF MONONUCLEAR CELLS

CD4+ T Cells The primary immunopathogenic lesion in HIV infection involves CD4+ T cells, and the range of CD4+ T cell abnormalities in advanced HIV infection is broad. The defects are both

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quantitative and qualitative and ultimately impact virtually every limb of the immune system, indicating the critical dependence of the integrity of the immune system on the inducer/helper function of CD4+ T cells. In advanced HIV disease, most of the observed immune defects can ultimately be explained by the quantitative depletion of CD4+ T cells. However, T cell dysfunction can be demonstrated in patients early in the course of infection, even when the CD4+ T cell count is in the low-normal range. The degree and spectrum of dysfunctions increase as the disease progresses, reflecting the range of CD4+ T cell functional heterogeneity, especially in lymphoid tissues. One of the first sites of intense HIV replication is in the GALT where CD4+ T_H17 cells reside; they are important for host defense against extracellular pathogens in the intestinal mucosa and help maintain the integrity of the gut epithelium. In HIV infection, they are depleted by direct and indirect effects of viral replication and cause loss of gut homeostasis and integrity, as well as a shift toward a T_H1 phenotype. Studies have shown that even after many years of cART, normalization of the CD4+ T cells in the GALT remains incomplete. In lymph nodes, HIV perturbs another important subset of the CD4+ helper T lineage, namely TF_H cells (see "Lymphoid Organs and HIV Pathogenesis," above). TF_H cells, which are derived either directly from naïve CD4+ T cells or from other T_H precursors, migrate into B follicles during germinal center reactions and provide help to antigen-specific B cells through cell–cell interactions and secretion of cytokines to which B cells respond, the most important of which is IL-21. As with T_H17 cells, TF_H cells are highly susceptible to HIV infection. However, in contrast to T_H17 and most other CD4+ T cell subsets, the number of TF_H cells is increased in lymph nodes of HIV-infected individuals, especially those who are viremic. It is unclear whether this increase is helpful to responding B cells, although the likely outcome is that the increase in numbers is detrimental to the quality of the humoral immune response against HIV (see "Immune Response to HIV," below). In addition, defects of central memory cells are a critical component of HIV immunopathogenesis. The progressive loss of antigen-specific CD4+ T cells has important implications for the control of HIV infection. In this regard, there is a correlation between the maintenance of HIV-specific CD4+ T cell proliferative responses and improved control of infection. Essentially every T cell function has been reported to be abnormal at some stage of HIV infection. Loss of polyfunctional HIV-specific CD4+ T cells, especially those that produce IL-2, occurs early in disease, whereas IFN-producing CD4+ T cells are maintained longer and do not correlate with control of HIV viremia. Other abnormalities include impaired expression of IL-2 receptors, defective IL-2 production, reduced expression of the IL-7 receptor (CD127), and a decreased proportion of CD4+ T cells that express CD28, a major co-stimulatory molecule necessary for the normal activation of T cells, which is also depleted as a result of aging. Cells lacking expression of CD28 do not respond normally to activation signals and may express markers of terminal activation including HLA-DR, CD38, and CD45RO. As mentioned above ("The Role of Immune Activation and Inflammation in HIV Pathogenesis"), a subset of CD4+ T cells referred to as *T regulatory cells*, or T-regs, may be involved in damping aberrant immune activation that propagates HIV replication. The presence of these T-reg cells correlates with lower viral loads and higher CD4+/CD8+ T cell ratios. A loss of this T-reg capability with advanced disease may be detrimental to the control of virus replication.

It is difficult to explain completely the profound immunodeficiency noted in HIV-infected individuals solely on the basis of direct infection and quantitative depletion of CD4+ T cells. This is particularly apparent during the early stages of HIV disease, when CD4+ T cell numbers may be only marginally decreased. In this regard, it is likely that CD4+ T cell dysfunction results from a combination of depletion of cells due to direct infection of the cell and a number of virus-related but indirect effects on the cell such as elimination of "innocent bystander cells" (Table 197-5). Several of these effects have been demonstrated ex vivo and/or by the analysis of cells isolated from the peripheral blood. Soluble viral proteins, particularly gp120, can bind with high affinity to the CD4 molecules on uninfected T cells and monocytes; in addition, virus and/or viral proteins can bind to DCs or FDCs. HIV-specific antibody can recognize these bound molecules and potentially collaborate in the elimination of the cells by ADCC. HIV envelope glycoproteins gp120 and gp160 manifest high-affinity binding to the CD4 molecule as well as to various chemokine receptors. Intracellular signals transduced by gp120 through both CD4 and CCR5/CXCR4 have been associated with a number of immunopathogenic processes including anergy, apoptosis, and abnormalities of cell trafficking. The molecular mechanisms responsible for these abnormalities include dysregulation of the T cell receptor–phosphoinositide pathway, p56lck activation, phosphorylation of focal adhesion kinase, activation of the MAP kinase and ras signaling pathways, and downregulation of the co-stimulatory molecules CD40 ligand and CD80.

The inexorable decline in CD4+ T cell counts that occurs in most HIV-infected individuals may result in part from the inability of the immune system to regenerate over an extended period of time the rapidly turning over CD4+ T cell pool efficiently enough to compensate for both HIV-mediated and naturally occurring attrition of cells. In this regard, the degree and duration of decline of CD4+ T cells at the time of initiation of therapy is an important predictor of the restoration of these cells. A person who maintains a very low CD4+ T cell count for a considerable period of time before the initiation of cART almost invariably has an incomplete reconstitution of such cells. At least two major mechanisms may contribute to the failure of the CD4+ T cell pool to reconstitute itself adequately over the course of HIV infection. The first is the destruction of lymphoid precursor cells, including thymic and bone marrow progenitor cells; the other is the gradual disruption of the lymphoid tissue architecture and microenvironment, which is essential for efficient regeneration of immunocompetent cells. Finally, during the advanced stages of CD4+ T lymphopenia, there are increased serum levels of the homeostatic cytokine IL-7. It was initially felt that this elevation was a homeostatic response to the lymphopenia; however, recent findings suggest that the increase in serum IL-7 was a result of reduced utilization of the cytokine related to the loss of cells expressing the IL-7 receptor, CD127, which serves as a normal physiologic regulator of IL-7 production.

CD8+ T Cells A relative CD8+ T lymphocytosis is generally associated with high levels of HIV plasma viremia and likely reflects an immune response to the virus as well as dysregulated homeostasis associated with generalized immune activation. During the late stages of HIV infection, there may be a significant reduction in the numbers of CD8+ T cells despite the presence of high levels of viremia. HIV-specific CD8+ CTLs have been demonstrated in HIV-infected individuals early in the course of disease, and their emergence often coincides with a decrease in plasma viremia—an observation that is a factor in the proposal that virus-specific CTLs can control HIV disease for a finite period of time in a certain percentage of infected individuals. However, emergence of HIV escape mutants that ultimately evade these HIV-specific CD8+ T cells has been described in the majority of HIV-infected individuals who are not receiving cART. In addition, as the disease progresses, the functional capability of these cells gradually decreases, at least in part due to the persistent nature of HIV infection that causes functional exhaustion via the upregulation of inhibitory receptors such as PD-1 and TIGIT on HIV-specific CD8+ T cells (see "The Role of Immune Activation and Inflammation in HIV Pathogenesis," above). As chronic immune activation persists, there are also systemic effects on CD8+ T cells, such that as a population they assume an abnormal phenotype characterized by expression of activation markers such as HLA-DR and CD38 with an absence of expression of the IL-2 receptor (CD25) and a reduced expression of the IL-7 receptor (CD127). In addition, CD8+ T cells lacking CD28 expression are increased in HIV disease, reflecting a skewed expansion of a less differentiated CD8+ T cell subset. This skewing of subsets is also associated with diminished polyfunctionality, a qualitative difference that distinguishes elite controllers from progressors. Elite controllers can also be distinguished from progressors by the maintenance in the former of a high proliferative capacity of their HIV-specific CD8+ T cells coupled to increases in perforin expression and elimination of infected targets, characteristics that are markedly diminished in advanced HIV disease. It has been

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reported that the phenotype of CD8+ T cells in HIV-infected individuals may be of prognostic significance. Those individuals whose CD8+ T cells developed a phenotype of HLA-DR+/CD38– following seroconversion had stabilization of their CD4+ T cell counts, whereas those whose CD8+ T cells developed a phenotype of HLA-DR+/CD38+ had a more aggressive course and a poorer prognosis. In addition to the defects in HIV-specific CD8+ CTLs, functional defects in other MHC-restricted CTLs, such as those directed against influenza and CMV, have been demonstrated. CD8+ T cells secrete a variety of soluble factors that inhibit HIV replication, including the CC-chemokines RANTES (CCL5), MIP-1α (CCL3), and MIP-1β (CCL4) as well as potentially a number of as yet unidentified factors. The presence of high levels of HIV viremia in vivo as well as exposure of CD8+ T cells in vitro to HIV envelope, both of which are associated with aberrant immune activation, have been shown to be associated with a variety of cellular functional abnormalities. Furthermore, since the integrity of CD8+ T cell function depends in part on adequate inductive signals from CD4+ T cells, the defect in CD8+ CTLs is likely compounded by the quantitative loss and qualitative dysfunction of CD4+ T cells.

B Cells The predominant defect in B cells from HIV-infected individuals is one of aberrant cellular activation, which is reflected by increased propensity to terminal differentiation and immunoglobulin secretion, as well as increased expression of markers of activation and exhaustion. As a result of activation and differentiation in vivo, B cells from HIV viremic patients manifest a decreased capacity to mount a proliferative response to ligation of the B cell antigen receptor and other B cell stimuli in vitro. B cells from HIV-infected individuals manifest enhanced spontaneous secretion of immunoglobulins in vitro, a process that reflects their highly differentiated state in vivo. There is also an increased incidence of EBV-related B cell lymphomas in HIV-infected individuals that are likely due to combined effects of defective T cell immune surveillance and increased B cell turnover that increases the risk of oncogenesis. Untransformed B cells cannot be infected with HIV, although HIV or its products can activate B cells directly. B cells from patients with high levels of viremia bind virions to their surface via the CD21 complement receptor. It is likely that in vivo activation of B cells by replication-competent or defective virus as well as viral products during the viremic state accounts at least in part for their activated phenotype. B cell subpopulations from HIV-infected individuals undergo a number of changes over the course of HIV disease, including the attrition of resting memory B cells and replacement with several aberrant memory and differentiated B cell subpopulations that collectively express reduced levels of CD21 and either increased expression of activation markers or inhibitory receptors associated with functional exhaustion. The more activated and differentiated B cells are also responsible for increased secretion of immunoglobulins and increased susceptibility to Fas-mediated apoptosis. In more advanced disease, there is also the appearance of immature B cells associated with CD4+ T cell lymphopenia. Despite increased frequencies of germinal center B cells and CD4+ TF_H cells, both of which are required for effective humoral immunity, cognate B cell–CD4+ T cell interactions in lymphoid tissues are perturbed in HIV-infected individuals, especially those with persistent viremia. In vivo, the aberrant activated state of B cells manifests itself by hypergammaglobulinemia and by the presence of circulating immune complexes and autoantibodies. HIV-infected individuals respond poorly to primary and secondary immunizations with protein and polysaccharide antigens. Using immunization with influenza vaccine, it has been demonstrated that there is a memory B cell defect in HIV-infected individuals, particularly those with high levels of HIV viremia. There is also evidence that responses to HIV and non-HIV antigens in infected individuals, especially those who remain viremic, are enriched in abnormal subsets of B cells that either are highly prone to apoptosis or show signs of functional exhaustion. Taken together, these B cell defects are likely responsible at least in part for the inadequate humoral response to HIV as well as to decreased response to vaccinations and the increase in certain bacterial infections seen in advanced HIV disease in adults. In addition, they likely contribute to the inadequacy of host defenses against bacterial infections that play a role in the increased morbidity and mortality of HIV-infected children. The absolute number of circulating B cells also may be depressed in HIV infection; this phenomenon likely reflects increased activation-induced apoptosis as well as a redistribution of cells out of the circulation and into the lymphoid tissue—phenomena that are associated with ongoing viral replication.

Monocytes/Macrophages Circulating monocytes are generally normal in number in HIV-infected individuals; however, there is evidence of increased activation within this lineage. The increased level of sCD14 and other biomarkers (see above) reported in HIV-infected individuals is an indirect marker of monocyte activation in vivo. A number of other abnormalities of circulating monocytes have been reported in HIV-infected individuals, many of which may be related directly or indirectly to aberrant in vivo immune activation. In this regard, increased levels of lipopolysaccharide (LPS) are found in the sera of HIV-infected individuals due, at least in part, to translocation across the gut mucosal barrier (see above). LPS is a highly inflammatory bacterial product that preferentially binds to macrophages through CD14 and Toll-like receptors, resulting in cellular activation. Functional abnormalities of monocyte/macrophages in HIV disease include decreased secretion of IL-1 and IL-12; increased secretion of cytokines such as IL-10 and IL-18 and markers of coagulation such as D-dimer; defects in antigen presentation and induction of T cell responses due to decreased MHC class II expression; and abnormalities of Fc receptor function, C3 receptor–mediated clearance, oxidative burst responses, and certain cytotoxic functions such as ADCC, possibly related to low levels of expression of Fc and complement receptors. Monocytes express the CD4 molecule and several co-receptors for HIV on their surface, and thus are potential targets of HIV infection. However, in vivo infection of circulating monocytes is difficult to demonstrate, although infection of tissue macrophages and macrophage-lineage cells in the brain (infiltrating macrophages or resident microglial cells) and lung (pulmonary alveolar macrophages) can be demonstrated easily. Tissue macrophages are an important source of HIV during the inflammatory response associated with opportunistic infections and can serve as persistent reservoirs of HIV infection, thus representing an obstacle to the eradication of HIV by antiretroviral drugs. Infection of monocyte precursors in the bone marrow may directly or indirectly be responsible for certain of the hematologic abnormalities in HIV-infected individuals. However, as with DCs, monocytes and macrophages express high levels of host restriction factors that likely help explain the low contribution of myeloid cells to the overall viral burden in HIV-infected individuals.

Dendritic and Langerhans Cells DCs and Langerhans cells are not productively infected with HIV, but they are thought to play an important role in the initiation of HIV infection by virtue of the ability of HIV to bind to cell-surface C-type lectin receptors, particularly DC-SIGN (see above) and langerin. However, while langerin provides a host barrier for replication by trafficking HIV to acidic compartments for degradation, DC-SIGN retains HIV in early endosomal compartments. This allows efficient presentation of intact virus to CD4+ T cell targets that become infected; complexes of infected CD4+ T cells and DCs provide an optimal microenvironment for virus replication. Furthermore, pDCs secrete large amounts of IFN-α in response to viral infections and as such play an important role in innate sensing of HIV during early phase of infection. The numbers of circulating pDCs are decreased in HIV infection through mechanisms that remain unclear, although several studies have shown increased lymphoid tissue recruitment of DCs associated with lymphoid hyperplasia and inflammation. The mDCs or conventional DCs are also involved in the initiation of adaptive immunity in draining lymph nodes by presenting antigen to T cells and B cells, as well as by secreting cytokines such as IL-12, IL-15, and IL-18 that activate other immune cells. There are also indications that the relatively low infectibility of DCs may be associated with the expression of host restriction factors, including APOBEC3G and SAMHD1 (see above).

Natural Killer Cells The role of NK cells is to provide immunosurveillance against virus-infected cells, certain tumor cells, and

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allogeneic cells (**Chap. 342**). There are no convincing data that HIV productively infects NK cells in vivo; however, functional abnormalities in NK cells have been observed throughout the course of HIV disease, and the severity of these abnormalities increases as disease progresses. NK cells are part of the innate immune system and act by direct killing of infected cells and secretion of antiviral cytokines and chemokines. In early HIV infection there is an increase in the activation of NK cells, and the capacity to secrete IFN-γ is maintained, although they manifest reduced cytotoxic function. During chronic HIV infection, both NK cell cytotoxicity and cytokine secretion become impaired. Given that HIV infection of target cells downregulates HLA-A and B, but not HLA-C and D molecules, this may explain in part the relative inability of NK cells to kill HIV-infected target cells. However, the NK cell impairments, especially in patients with high levels of virus replication, are associated with an expansion of an "anergic" CD56–/CD16+ NK cell subset. This abnormal subset of NK cells manifests an increased expression of inhibitory NK cell receptors (iNKRs) and a substantial decrease in expression of natural cytotoxicity receptors (NCRs) and shows a markedly impaired lytic activity. The overrepresentation of this abnormal subset of NK cells may explain in part the observed defects in NK cell function in HIV-infected individuals and likely begins to occur during primary infection. The relative expression of iNKRs and NCRs—as well as their ligands, which include HLA class I molecules—has an impact on the antiviral functions associated with NK cells, including direct killing and ADCC. Polymorphisms in iNKR and NCR alleles have been linked to HIV-1 disease outcomes. NK cells also serve as important sources of HIV-inhibitory CC-chemokines. NK cells isolated from HIV-infected individuals constitutively produce high levels of MIP-1α (CCL3), MIP-1β (CCL4), and RANTES (CCL5), although the impact of these chemokines on HIV replication in vivo is unclear. Finally, NK cell–DC interactions are important for normal immune function. NK cells and DCs reciprocally modulate each other's activation and maturation. These interactions are markedly impaired in HIV-infected individuals with high levels of plasma viremia.

GENETIC FACTORS IN HIV-1 AND AIDS PATHOGENESIS

Candidate gene approaches and genome-wide association studies (GWAS) have identified polymorphisms in host genes that contribute to inter-individual variation in (1) the risk of acquiring HIV, (2) the steady-state levels of HIV that are established soon after infection (virologic set point), (3) the rate at which untreated HIV-infected patients progress to AIDS as well as risk of developing specific AIDS-defining illnesses (e.g., renal and neurologic diseases), and (4) the level of immune reconstitution (e.g., CD4+ counts) achieved after initiation of virally suppressive ART. The key polymorphisms that influence these four traits are summarized in **Table 197-6**, and their identification has greatly advanced our understanding of the genes that influence HIV-AIDS pathogenesis. Of particular interest are polymorphisms in two chromosomal regions, as they are associated with consistent effects on HIV acquisition, virologic set point, and/or rates of HIV disease progression: the region in chromosome 3 that includes the gene that encodes the HIV co-receptor *CC chemokine receptor 5* (*CCR5*) and the *major histocompatibility locus* (MHC) in chromosome 6 (**Fig. 197-26**).

GENETICS OF CCR5: FROM BENCH TO BEDSIDE While the discovery of CCR5 as a major co-receptor for cell entry of HIV-1 was established by in vitro studies, genetic association studies were required to establish its seminal role in HIV pathogenesis. Initial in vitro studies revealed that a 32-bp deletion (*Δ32*) in the coding region of CCR5 contributes to resistance to CCR5-using R5 strains of HIV. The *CCR5 Δ32* allele encodes a truncated protein that is not expressed on the cell surface. Congruently, genotype-phenotype association studies in large cohorts demonstrated that individuals homozygous for the *CCR5 Δ32* allele (*Δ32/Δ32*) lack CCR5 surface expression and are highly resistant to acquiring HIV infection; heterozygosity for the *CCR5 Δ32* allele is associated with a lower risk of acquiring HIV.

The distribution of the *CCR5 Δ32* allele is population specific. Approximately 1% of individuals of European ancestry are homozygous for the *CCR5 Δ32* allele. Depending on the geographic region in Europe, up to 18% of individuals are heterozygous for the *CCR5 Δ32* allele. The *CCR5 Δ32* allele is rare in other populations. The evolutionary pressure that resulted in the emergence of the *CCR5 Δ32* allele in the European population remains unknown and has been speculated to be secondary to an ancestral pandemic, such as the plague.

Subsequent studies identified single nucleotide polymorphisms (SNPs) in the promoter (regulatory) region of *CCR5* that influence gene expression levels. Alleles bearing specific cassettes of linked polymorphisms (haplotypes) were identified and designated as human haplogroups A to G*2 (HHA to HHG*2) (Fig. 197-26). The *CCR5 Δ32* polymorphism is found on the HHG*2 haplotype. *CCR5* haplotypes A–D vs. E–G*2 differ by bearing GT versus AC at polymorphic sites rs1799987 and rs1799988 (Fig. 197-26). *CCR5-HHA* haplotype represents the ancestral haplotype (found in chimpanzees) and is associated with lower CCR5 gene expression, whereas the *CCR5-HHE* haplotype is associated with higher CCR5 expression. Methylation of DNA is a common epigenetic signaling mechanism that cells use to lock genes in the "off" position, and polymorphisms in *CCR5* haplotypes may mediate their effects by influencing DNA methylation levels in the *CCR5* locus. The *CCR5-HHE* and *CCR5-HHA* haplotypes are more sensitive and resistant, respectively, to T cell activation–induced demethylation of the *CCR5* locus.

In worldwide populations, HHE and HHC are more prevalent, whereas the ancestral HHA haplotype is more common in persons of African ancestry. The associations of *CCR5* haplotypes with HIV acquisition and/or HIV disease course are largely consistent with their effects on *CCR5* gene expression. For example, homozygosity for the *CCR5-HHE* haplotype is associated with an increased risk of acquiring HIV, progressing rapidly to AIDS, and reduced immune recovery while the patient is on ART. The HHA haplotype is associated with slower disease progression in African populations and has been speculated to be a basis for why chimpanzees (who all carry the ancestral *CCR5* HHA haplotype) naturally infected with simian immunodeficiency virus (SIV) may resist disease progression. The pairing of the HHC and *CCR5 Δ32*-bearing HHG*2 haplotypes (HHC/HHG*2 genotype) is associated with a lower risk of acquiring HIV infection and slower rate of HIV disease progression, whereas the pairing of the HHE haplotype with the HHG*2 haplotype is associated with the opposite effects. The *CCR2*-64I-bearing HHF*2 haplotype is associated with a slower HIV disease course.

Consistent with these genetic associations, polymorphisms in genes encoding ligands for CCR5 have also been demonstrated to associate with variable HIV susceptibility and disease progression rates. Examples include copy number variations of *CCL3L1* and SNPs in *CCL5*. The sum of these studies established a pivotal role of CCR5 and its ligands in HIV-AIDS pathogenesis and, potentially, immune recovery.

The discovery that the *CCR5 Δ32/Δ32* genotype is associated with strong resistance to HIV infection, and that uninfected Caucasians bearing this genotype did not appear to have impaired immunity, led to the development of two kinds of novel therapies. First, it spurred the development of a new class of therapies approved by the U.S. Food and Drug Administration (FDA), i.e., entry inhibitors (e.g., maraviroc) that block the interaction of CCR5 with the HIV envelope. Second, it led to the evaluation of novel experimental cellular therapies. An HIV-infected patient with acute myelogenous leukemia was given an allogeneic stem cell transplantation from an HLA-compatible person whose cells lacked expression of CCR5 due to the *Δ32/Δ32* genotype. There has been no evidence of HIV-1 infection in the patient who underwent the transplant thus far (~10 years). This observation provided a "proof of concept" for an HIV cure and led to the development of additional novel cellular therapies involving autologous transplantation of CD4+ T cells in which the *CCR5* gene is inactivated ex vivo using new gene editing procedures.

DISCOVERY OF HLA CLASS I ALLELES THAT ASSOCIATE WITH VIROLOGIC CONTROL OF HIV INFECTION There is a strong association between variations within the *HLA-B* gene with protective (e.g., HLA-B*57 and B*27 alleles) or detrimental (e.g., HLA-B*35 allele) outcomes during

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TABLE 197-6 Host Genetic Factors That Influence Risk of HIV-1 Acquisition and Rates of HIV-1 Disease Progression

GENE[a]	GENETIC VARIATION	MECHANISMS[b]	GENETIC EFFECT ON HIV-AIDS[c]
Genes in MHC Locus			
HLA-B	B*27 and B*57	Presentation of specific HIV antigens	Slower progression to AIDS; lower viral load
	B*35	Restriction of specific HIV peptide presentation	Faster progression to AIDS; higher viral load
	HLA-Bw4	Providing ligands for activating KIR	Slower progression to AIDS
HLA class I allele	Homozygosity of *HLA*-class I alleles	Reduced repertoire for epitope recognition	Faster progression to AIDS; increased risk of mother-to-child transmission
	Shared donor-recipient *HLA* alleles	Preadaptation of HIV strains	Faster disease progression to AIDS
	Rare *HLA* alleles	Limited adaptation of HIV strains; less frequent escape mutants	Protection against HIV infection
HLA class II allele	*HLA-DRB1* alleles	Influencing protein specificity of CD4+ T cell responses to HIV Gag and Nef proteins	HLA-DRB1*15:02—lower viral load; HLA-DRB1*03:01—higher viral load
HLA extended haplotype	A1-B8-DR3-DQ2 (AH 8.1)	Increased proinflammatory responses; higher TNF-α production	Faster progression to AIDS
HLA-C	35 kb upstream, rs9264942-C	Increased expression of HLA-C	Decreased viral load set point
HCP5	rs2395029-G	Linkage disequilibrium with HLA-B*57:01	Lower viral load
MICA	Noncoding SNP near *MICA*, rs4418214-T	May affect HLA class I peptide presentation—linkage with protective *HLA-B* alleles	Enriched in HIV-1 controllers
PSORS1C3	rs3131018-A	May affect HLA class I peptide presentation	Enriched in HIV-1 controllers
ZNRD1	rs9261174-C	Possible interference in processing of HIV transcripts; influencing ZNRD1 expression; linkage disequilibrium with *HLA-A10*	Slower disease progression to AIDS
Chemokine Receptors			
CCR5	32-bp deletion in the ORF (Δ32), rs333	Truncated CCR5 protein	Δ32/Δ32: resistance to acquiring HIV infection
			Δ32/wild type: delays AIDS onset; improves immune reconstitution during ART
	Promoter SNPs, haplotypes (HHA to HHG*2)	Altered CCR5 expression, e.g., HHE haplotype correlates with high CCR5 expression	HHE/HHE: increased HIV/AIDS susceptibility
CCR2	SNP in ORF (64 V→I), rs1799864	Possibly due to linkage with polymorphism in *CCR5* promoter	64I: delayed AIDS onset
CCRL2	Coding SNP (167 Y→F) rs3204849	Possibly due to linkage with *CCR5* haplotype	167F is associated with accelerated progression to AIDS and more rapid development of PCP
	rs1015164	Possibly due to linkage with *CCR5* haplotype	Associated with high viral load set point
CXCR6	rs2234358 G→T in the 3'UTR	Trafficking of effector T cells and activation of NK T cells; minor HIV co-receptor	Prevalence of rs2234358-T was lower in long-term nonprogressors and viremic controllers
CX3CR1	SNPs in ORF (249 V→I, rs3732379; and 280 T→M, rs3732378)	280M reduces receptor expression and binding of fractalkine, the CX3CR1 ligand	249I and 280M associated with faster AIDS onset in some Caucasian cohorts; inconsistent effects detected in other cohorts
DARC	African-specific promoter SNP (–46T→C), rs2814778	–46C/C associates with low neutrophil counts; influences circulating chemokine levels; alters HIV binding to RBCs and transinfection of HIV-1	–46C/C: increased risk of acquiring HIV but slower HIV disease progression; Duffy-null-associated low neutrophil trait associated with increased HIV risk in persons of African descent
Chemokines			
CCL3L, CCL4L	Gene copy number of *CCL3L* and *CCL4L*	High numbers of *CCL3L* and *CCL4L* gene-containing segmental duplications correlate with high CCL3L and CCL4L levels	Gene copy number lower than population median associated with increased HIV/AIDS susceptibility and reduced immune reconstitution during ART
CCL5	Promoter SNPs	Altered gene expression	Altered HIV-AIDS susceptibility
CCL2	Promoter SNP (–2578 T→G), rs1024611	–2578G allele: increased CCL2 expression and monocyte recruitment	–2578G/G associated with increased risk of developing HIV-1-associated dementia and rapid AIDS onset
Cytokines			
IL-6	Promoter SNP (–174 G→C), rs1800795	–174G/G associated with increased IL-6 and CRP levels	–174G/G associated with high risk of KS development and variable recovery of CD4 cells during ART
IL-7RA	Coding SNP (244 T→I), rs6897932	244 I/I associated with increased signal transduction and proliferation in response to IL-7	244 I/I associated with faster CD4+ T cell recovery after ART initiation
IL-10	Promoter SNP (–592 C→A), rs1800872	–592A results in decreased IL-10 levels	–592A associated with increased HIV-AIDS susceptibility

(*Continued*)

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TABLE 197-6 Host Genetic Factors That Influence Risk of HIV-1 Acquisition and Rates of HIV-1 Disease Progression (Continued)

GENE[a]	GENETIC VARIATION	MECHANISMS[b]	GENETIC EFFECT ON HIV-AIDS[c]
Innate Immunity Genes			
MBL	Coding alleles (O)	Low plasma concentration and structural variation of MBL protein	Slow progression to AIDS in heterozygous subjects (A/O)
	X allele (promoter SNP –221)	Decreased levels of MBL protein	Faster progression to AIDS in homozygous X/X subjects
Apobec-3G	ORF SNP (186 H→R), rs8177832	Reduced anti-HIV-1 activity	186R associated with rapid AIDS onset in African Americans
Apobec-3F	Haplotype tagged by ORF SNP (231 I→V), rs2076101	231V variant may influence Vif-mediated Apobec-3F degradation	231V associated with lower VL, slower progression to AIDS and delayed progression to PCP
TLR7	ORF SNP (Gln11Leu), rs179008	Decreased expression of TLR7 leading to lack of recognition of HIV-infected cells	Leu-containing protein associated with higher viral load and faster progression to AIDS
PARD3B	rs11884476 (C→G), near exon 20	Direct interaction with HIV, signaling through SMAD family of proteins	rs11884476-G associated with slower progression to AIDS
IFNL4	Frameshift mutation (TT→ΔG), rs368234815	Functional polymorphism in *IFNL4* gene, possibly in linkage with IL28B variant and regulates IL-28B levels	rs368234815-ΔG associated with higher prevalence of AIDS-defining illnesses and potentially increased HIV-1 infection risk
Others			
ApoE	E4 allele	E4 enhances HIV cell entry in vitro	ApoE4/E4 associated with rapid AIDS onset and dementia
ApoL1/ MYH9	Several risk haplotypes, including G1	Unknown	Increased risk for HIV-associated nephropathy
RYR3	ORF SNP (A →G), rs2229116	Unknown, potential impact on calcium signaling and homeostasis	rs2229116-G associated with subclinical atherosclerosis
PROX1	rs17762192-G, 36kb upstream of *PROX1*	Unknown, presumably due to its impact on PROX1 expression, which is a negative regulator of IFN-γ	rs17762192-G: reduced rate of disease progression
Gene–Gene Interaction			
KIR+HLA	KIR3DS1 + HLA-Bw4-80Ile	Altered NK cell activity required to eliminate HIV-infected cells	DS1 associated with HLA-Bw4-80Ile; +: ed AIDS onset
	HLA-C1 + KIR2DL3	Reduction of inhibitory KIR likely results in increased immune activation; impaired killing of latently infected cells; and a higher proviral burden	HLA-C1+/KIR2DL3+: better immune recovery after viral load suppression on ART
LILRB2+HLA	LILRB2 + HLA class I	Regulation of dendritic cells by LILRB2-HLA engagement	Control of HIV-1
CCL3L1+ CCR5	Low *CCL3L1* gene copies + detrimental *CCR5* genotypes	Low CCL3L1 and high CCR5 expression	Increased HIV/AIDS susceptibility and reduced immune reconstitution during ART

[a]Representative genes and polymorphisms and [b]possible mechanisms are listed. [c]Some of the associations are population specific and may display cohort-specific effects.

Note: Apobec, apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like; ApoE, apolipoprotein E; ART, antiretroviral therapy; CCL, CC ligand; CCL3L, CCL3-like; CCR5, CC chemokine receptor 5; CCRL2: CC chemokine receptor like 2; CRP, C-reactive protein; CXCR6, chemokine (C-X-C motif) receptor 6; DARC, Duffy antigen receptor for chemokines; HCP5, HLA class I histocompatibility antigen protein P5; HHE, human haplogroup E; HLA, human leukocyte antigen; IFN, interferon; IFNL4, interferon λ4 gene; IL, interleukin; IL-7RA, interleukin 7 receptor-α; KIR, killer cell immunoglobulin-like receptors; KS, Kaposi's sarcoma; LILRB2, leukocyte immunoglobulin-like receptor B2; MBL, mannose-binding lectin; MHC, major histocompatibility complex; MICA, MHC class I polypeptide-related sequence A; NK, natural killer; ORF, open reading frame; PARD3B, par-3 family cell polarity regulator beta; PCP, *Pneumocystis* pneumonia; PROX1, prospero homeobox 1; PSORS1C3, psoriasis susceptibility 1 candidate 3; SMAD, Mothers against decapentaplegic homolog; SNP, single nucleotide polymorphism; rs#, SNP identification number; TNF-α, tumor necrosis factor-α; UTR, untranslated region; VL, viral load; ZNRD1, zinc ribbon domain containing 1; +, present, –, absent.

Sources: Sunil K Ahuja, MD, Weijing He, MD, Kristen Rogers, MS. Reviews for additional information: P An et al: Trends Genet 26:119, 2010; J Fellay: Antivir Ther 14:731, 2009; RA Kaslow et al: J Infect Dis 191:S68, 2005; D van Manen et al: Retrovirology 9:70, 2012; MP Martin et al: Immunol Rev 254:245, 2013; S Limou et al: Front Immunol 4:118, 2013; PJ McLaren et al: Curr Opin HIV AIDS 10:110, 2015; PJ McLaren et al: Proc Natl Acad Sci USA 112:14658, 2015; PJ McLaren, M Carrington: Nat Immunol 16:577, 2015; P An et al: PLoS Genet 12:e1005921, 2016; F Pereyra et al: Science 330:1551, 2010; I Bartha et al: PLoS Comput Biol 13:e1005339, 2017.

HIV infection. Carriage of the HLA-B*57 and/or HLA-B*27 alleles is associated with slower disease progression. The beneficial effects of these alleles may relate in part to their consistent associations with a lower virologic set point as well as to higher cell-mediated immunity in HIV-infected persons. The protective effect of the HLA-B*57 and B*27 alleles on the HIV disease course is underscored by the finding that the prevalence of these alleles is higher among long-term nonprogressors and persons who control HIV replication spontaneously (elite controllers). In contrast, the HLA-B*35 allele has been associated with faster progression to AIDS and higher viral load. The prevalence of the *HLA-B* alleles differs between populations. HLA-B*57:01 in Europeans and HLA-B*57:03 in African Americans are the protective alleles. In some populations (e.g., Japanese) where the HLA-B*57/-B*27 alleles are absent, HLA-B*51 is associated with a protective phenotype.

Possession of the protective *HLA-B* alleles is associated with broader and stronger CD8+ T cell responses to HIV epitopes. The mechanisms underlying the differential effects of the *HLA-B* alleles on the course of HIV disease may relate to differences in the ability of antigen-presenting cells to present immunodominant HIV epitopes to T helper or cytotoxic T lymphocytes in the context of MHC-encoded molecules. This may result in differential immune responses that influence viral replication. In this regard, the *HLA-B* alleles that impact the course of HIV disease differ in their amino acid residues in the HLA-B peptide-binding groove; this may play a critical role in virologic control.

Investigators have also examined the influence of extended *HLA* haplotypes (linked alleles) on the course of HIV disease. The extended *HLA* ancestral haplotype (AH) 8.1 is defined by the presence of HLA-A1, HLA-B8, and HLA-DR3 alleles. AH 8.1 is the most common ancestral haplotype in Caucasians (present in 10%) and is associated with multiple autoimmune diseases in HIV-uninfected persons. These associations of AH 8.1 are thought to be due to a genetically determined hyperresponsiveness characterized by high TNF-α production

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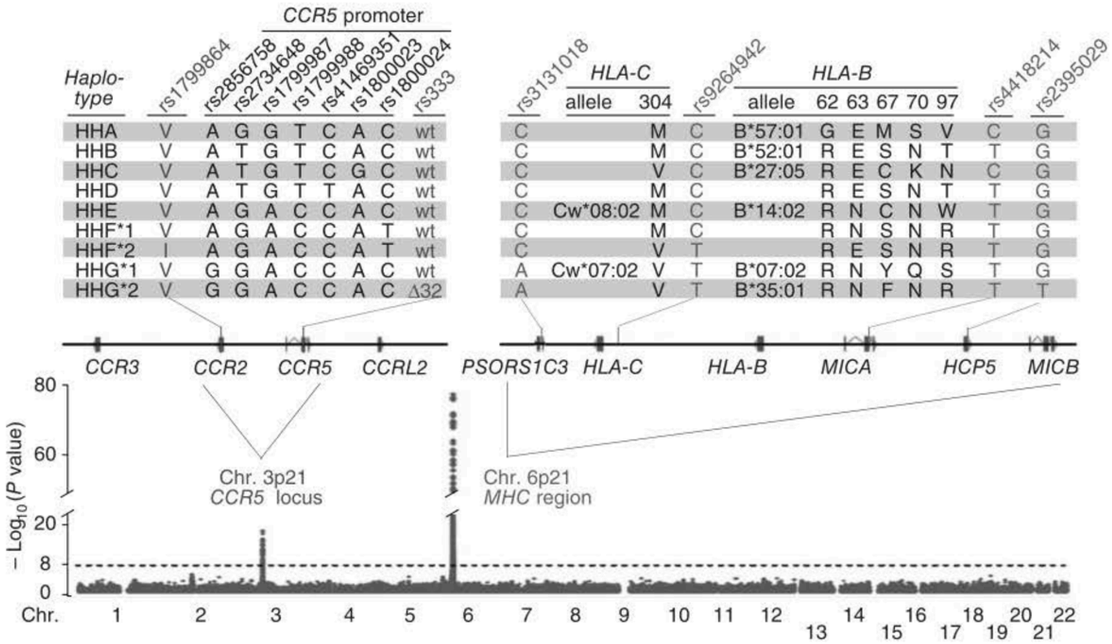

FIGURE 197-26 CCR5 and MHC loci: two key regions that influence HIV-AIDS pathogenesis. ***Bottom:*** Manhattan plot of genome-wide association from >6000 HIV-infected Europeans. *(Adapted from PJ McLaren et al: Proc Acad Natl Acad Sci 112:14658, 2015.)* ~8 million common variants were tested for their association with HIV viral load set point using linear regression. Genome-wide signals of association ($p < 5 \times 10^{-8}$, dotted line) were observed on chromosomes (Chr.) 6p21 in the MHC region and 3p21 in the *CCR5* locus. ***Upper left:*** schema depicting composition of *CCR5* haplotypes. *(Adapted from E Gonzalez et al: Proc Natl Acad Sci USA 96:12004, 1999.)* The 9 human haplotypes (HH) designated as HHA to HHG*2 were derived from the coding polymorphism of CCR2 64 V → I; 7 single nucleotide polymorphisms (SNPs) in the *CCR5* promoter region as well as the Δ32 coding mutation in *CCR5*; wt, wild type. Carrington and colleagues refer to the *CCR5-HHE* haplotype as the P1 haplotype (*MP Martin et al: Science 282:1907, 1998*). ***Upper right:*** Representative haplotypes at Chr. 6p21 MHC region in Europeans. *(Adapted from F Pereyra et al: Science 330:1551, 2010.)* Haplotypes defined by the SNPs identified by genome-wide association studies, classic HLA alleles and amino acids in HLA-B and HLA-C that are associated with HIV viral control. Figure is not to scale and full references are in Table 197-6.

and lack of complement C4A. Strong epidemiologic data indicate that carriage of AH 8.1 in HIV-infected persons is associated with a rapid decline in CD4+ T cells and faster progression to AIDS development. Gene–gene interactions between HLA alleles and other genes (e.g., killer cell immunoglobulin-like receptors) also may influence HIV disease progression rates.

POLYMORPHISMS IDENTIFIED BY GWAS THAT ASSOCIATE WITH HIV-1 ACQUSITION AND VIROLOGIC CONTROL GWAS have not identified additional genetic variations that associate with risk of HIV-1 acquisition. By contrast, large-scale GWAS have identified SNPs, especially in the MHC, that influence HIV viral load, including in a large group of individuals termed "HIV controllers" who spontaneously (without ART) control viral replication. GWAS in HIV-infected persons of European ancestry identified four SNPs in genes in the HLA class I loci that associated with virologic control. These SNPs are within or in the vicinity of *PSORS1C3*, *HLA-C*, *MICA*, and *HCP5* genes (Fig. 197-26). As noted in this figure, the individual effects of these alleles are difficult to discern because of linkage disequilibrium. The protective effects of the SNPs in *HCP5* and *MICA* may relate to their linkage with known protective *HLA-B* alleles. The protective *HCP5* allele is in linkage disequilibrium with the HLA-B*57:01 allele, and the protective *MICA* allele tags with the HLA-B*57:01 and B*27:05 alleles. The protective *HLA-C* SNP is associated with higher HLA-C expression, and this effect is thought to be due to the altered binding of a microRNA to the *HLA-C* mRNA. Higher HLA-C expression has been associated with beneficial HIV phenotypes. The mechanism associated with the SNP in *PSORS1C3* is unknown. GWAS in African Americans identified a SNP that tags the HLA-B*57:03 allele that is known to associate with a lower virologic set point and slower disease course. Together, these GWAS data underscore the importance of variations in HLA class I loci in control of viral replication. A recent GWAS study suggested that an allele in the gene encoding *CCRL2* influences the HIV viral load set point. *CCRL2* is on chromosome 3p21 and resides ~30 kb downstream of the *CCR5* loci; its effect could potentially be due to its linkage with the *CCR5* haplotypes. Mathematical modeling revealed that variations in host genes may explain about 10% of the observed variability in HIV viral load, whereas viral genetic diversity may explain 29% of the variability.

GENETIC ASSOCIATIONS WITH SPECIFIC AIDS AND NON-AIDS CONDITIONS

Carotid artery disease Many of the non-AIDS events in HIV-infected individuals resemble those related to immune senescence and those found in the HIV-uninfected aging population. A functional SNP in the ryanodine receptor 3 (*RYR3*) gene was found to be associated with an increased risk of common carotid intima–media thickness (cIMT), which is a surrogate for subclinical atherosclerosis. Functional studies on *RYR3* and its isoforms demonstrate a major role of these receptors in modulating endothelial function and atherogenesis via calcium signaling pathways, providing a biologically plausible mechanism by which the SNP in *RYR3* may associate with increased cIMT risk.

Renal disease HIV-1-associated nephropathy (HIVAN) is a form of focal sclerosing glomerulonephritis caused by direct infection of kidney epithelial cells with HIV. HIVAN is more common in persons of African descent. There is evidence that polymorphisms in the *MYH9* gene and in the neighboring *APOL1* gene are a strong determinant of susceptibility to HIVAN in African Americans. The effect of carrying two *APOL1* risk alleles explains nearly 35% of HIVAN. The mechanisms by which *MYH9*/*APOL1* variants predispose to HIVAN are currently unknown.

HIV-associated neurocognitive disorder HIV-associated neurocognitive disorder (HAND) comprises a spectrum of neurocognitive deficits due to HIV infection. Variations in the apolipoprotein E (ApoE) gene have strong associations with Alzheimer's disease in the HIV-uninfected population. In HIV-infected persons, possession of the *ApoE4* allele has been associated with several cognitive outcomes, including dementia, peripheral neuropathy, and impairment in cognition and immediate and delayed verbal memory. Macrophage recruitment and activation play a central role in the development of many of the HAND syndromes. Variations in chemokines that play

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an influential role in macrophage activation and recruitment, namely *CCL2* (MCP-1) and *CCL3* (MIP-1α), have been shown to alter the risk of developing HAND. Variations in mitochondrial genes also have been associated with risk of AIDS and HAND. A GWAS identified a polymorphism in chromosome 14 in the T cell receptor α locus that may influence neurocognitive outcomes.

HIV-1 associated Pneumocystis pneumonia Human Apobec3 cytidine deaminases are intrinsic resistance factors to HIV-1. However, HIV-1 encodes a viral infectivity factor (Vif) that degrades Apobec3 proteins. Association studies suggest a role of the genetic variation in the Apobec3 family in HIV disease. A common haplotype derived from 6 SNPs in the *Apobec-3F* gene and tagged by a codon-changing variant is associated with significantly lower viral load set point, slower rate of progression to AIDS, and delayed development of *Pneumocystis* pneumonia (PCP). In addition, a coding SNP in the *CCRL2* gene is associated with accelerated progression to AIDS and more rapid development of PCP.

Associations with ART-related adverse events Abacavir, an effective antiretroviral agent, is associated with significant risk of hypersensitivity reactions (2–9% of cases). Interestingly, while the *HLA-B*57:01* allele is associated with a slower HIV disease course, possession of this allele is associated with a higher risk of abacavir-associated hypersensitivity. Pharmacogenetic screening for the *HLA-B*57:01* allele is recommended before initiation of abacavir treatment.

NEUROPATHOGENESIS IN HIV DISEASE

While there has been a remarkable decrease in the incidence of the severe forms of HIV encephalopathy among those with access to treatment in the era of effective cART, HIV-infected individuals can still experience milder forms of neurocognitive impairment despite adequate cART. Factors that contribute to the neurocognitive decline include lack of complete control of HIV replication in the brain; production of HIV proteins that may be neurotoxic; low CD4+ T cell nadir; chronic immune activation; comorbidities such as drug abuse, microvascular disease, older age, and diabetes; and the potential for neurotoxicity of certain antiretroviral drugs. HIV has been demonstrated in the brain and CSF of infected individuals with and without neuropsychiatric abnormalities. As opposed to lymphoid tissues, there are no resident lymphocytes in the brain. The main cell types that are infected in the brain in vivo are the perivascular macrophages and the microglial cells, which can sometimes form syncytia resulting in multinucleated giant cells; low-level viral replication is also seen in perivascular astrocytes. It has been proposed that monocytes that have already been infected in the blood can migrate into the brain, where they then reside as macrophages, or macrophages can be directly infected while residing within the brain. The precise mechanisms whereby HIV enters the brain are unclear; however, they are thought to relate, at least in part, to the ability of virus-infected and immune-activated macrophages to induce adhesion molecules such as E-selectin and vascular cell adhesion molecule 1 (VCAM-1) on brain endothelium. Other studies have demonstrated that HIV gp120 enhances the expression of intercellular adhesion molecule 1 (ICAM-1) in glial cells and HIV Tat protein can disrupt the tight junctions of the brain endothelial cells to facilitate entry of HIV-infected cells into the CNS. Virus isolates from the brain are preferentially R5 strains as opposed to X4 strains; in this regard, HIV-infected individuals who are heterozygous for *CCR5-Δ32* appear to be relatively protected against the development of HIV encephalopathy. Once HIV enters the brain due to pressures of the local environment, it evolves to develop distinct sequences in the env, tat, and LTR genes. These unique sequences have been associated with neurocognitive dysfunction; however, it is unclear if they are causal (see below).

HIV-infected individuals may manifest white matter lesions as well as neuronal loss. The white matter lesions are due to axonal injury and a disruption of the blood-brain barrier and not due to demyelination. Given the absence of evidence of HIV infection of neurons, HIV-mediated effects on neurons are thought to involve indirect pathways whereby viral proteins, particularly gp120 and Tat, trigger the release of endogenous neurotoxins from macrophages and to a lesser extent from astrocytes. In addition, it has been demonstrated that both HIV-1 Nef and Tat can induce chemotaxis of leukocytes, including monocytes, into the CNS. Neurotoxins can be released from monocytes as a consequence of infection and/or immune activation. Monocyte-derived neurotoxic factors have been reported to kill neurons via a variety of mechanisms including activation of the *N*-methyl-D-aspartate (NMDA) receptors and induction of oxidative stress. In addition, HIV gp120 shed by virus-infected monocytes could cause neurotoxicity by antagonizing the function of vasoactive intestinal peptide (VIP), by elevating intracellular calcium levels, and by decreasing neurotrophic factor levels in the cerebral cortex. A variety of monocyte-derived cytokines can contribute directly or indirectly to the neurotoxic effects in HIV infection; these include TNF-α, IL-1, IL-6, TGF-β, IFN-γ, platelet-activating factor, and endothelin. Furthermore, among the CC-chemokines, elevated levels of monocyte chemotactic protein-1 (MCP-1 or CCL-2) in the brain and CSF have been shown to correlate best with the presence and degree of HIV encephalopathy in ART-naïve patients. In addition, infection and/or activation of monocyte-lineage cells can result in increased production of eicosanoids, quinolinic acid, nitric oxide, excitatory amino acids such as L-cysteine and glutamate, arachidonic acid, platelet activating factor, free radicals, TNF-α, and TGF-β, which may contribute to neurotoxicity. Astrocytes may play diverse roles in HIV neuropathogenesis. Reactive gliosis or astrocytosis has been demonstrated in the brains of HIV-infected individuals, and TNF-α and IL-6 have been shown to induce astrocyte proliferation. In addition, astrocyte-derived IL-6 can induce HIV expression in infected cells in vitro. Furthermore, it has been suggested that astrocytes may downregulate macrophage-produced neurotoxins. Evidence of neuronal injury can be demonstrated by measuring neurofilament levels in CSF. Treatment with cART leads to improvement in neuropsychiatric manifestations and a decrease in these cytokine levels in CSF, suggesting that they are driven by the virus or by its products. However, even in patients on long-term cART, there may be evidence of persistently activated lymphocytes in the CSF. It is unclear if these lymphocytes may contribute to neuronal injury in the brain or are critical for controlling the CNS viral reservoir. However, some individuals may develop a subacute encephalitis due to an IRIS reaction (see below). This often occurs weeks or a few months after initiation of cART in individuals with low CD4+ T cell counts. It is thought that the recovery of CD4+ T cells causes a lymphocyte response to the CNS HIV reservoir. The contribution of host genetic factors to development of neuropsychiatric manifestations of HIV infection has not been well studied. However, evidence supports the role of several genetic factors including the E4 allele for apoE in an increased risk of HIV-associated neurocognitive disorders and peripheral neuropathy.

It has also been suggested that the CNS may serve as a relatively sequestered site for a reservoir of latently infected cells that might be a barrier for the eradication of virus by cART (see "The HIV Reservoir: Obstacles to the Eradication of Virus," above).

PATHOGENESIS OF KAPOSI'S SARCOMA

There are at least four distinct epidemiologic forms of KS: (1) the classic form that occurs in older men of predominantly Mediterranean or eastern European Jewish backgrounds with no recognized contributing factors; (2) the equatorial African form that occurs in all ages, also without any recognized precipitating factors; (3) the form associated with organ transplantation and its attendant iatrogenic immunosuppressed state; and (4) the form associated with HIV-1 infection. In the latter two forms, KS is an opportunistic disease; in HIV-infected individuals, unlike typical opportunistic infections, its occurrence is not strictly related to the level of depression of CD4+ T cell counts. The pathogenesis of KS is complex; fundamentally, it is an angioproliferative disease that is not a true neoplastic sarcoma, at least not in its early stages. It is a manifestation of excessive proliferation of spindle cells that are believed to be of vascular origin and have features in common with endothelial and smooth-muscle cells. In HIV disease the development of KS is dependent on the interplay of a variety of factors including HIV-1 itself, human herpes virus 8 (HHV-8), immune activation, and

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cytokine secretion. A number of epidemiologic and virologic studies have clearly linked HHV-8, which is also referred to as *Kaposi's sarcoma–associated herpesvirus* (KSHV), to KS not only in HIV-infected individuals but also in individuals with the other forms of KS. HHV-8 is a γ-herpesvirus related to EBV and *herpesvirus saimiri*. It encodes a homologue to human IL-6 and, in addition to KS, has been implicated in the pathogenesis of body cavity lymphoma, multiple myeloma, and monoclonal gammopathy of undetermined significance. Sequences of HHV-8 are found universally in the lesions of KS, and patients with KS are virtually all seropositive for HHV-8. HHV-8 DNA sequences can be found in the B cells of 30–50% of patients with KS and 7% of patients with AIDS without clinically apparent KS.

Between 1% and 2% of eligible blood donors are positive for antibodies to HHV-8, while the prevalence of HHV-8 seropositivity in HIV-infected men is 30–35%. The prevalence of HHV-8 seropositivity in HIV-infected women is ~4%. This finding is reflective of the lower incidence of KS in women. It has been debated whether HHV-8 is actually the transforming agent in KS; the bulk of the cells in the tumor lesions of KS are not neoplastic cells. However, it has been demonstrated that endothelial cells can be transformed in vitro by HHV-8. In this regard, HHV-8 possesses a number of genes, including homologues of the IL-8 receptor, Bcl-2, and cyclin D, that can potentially transform the host cell. Despite the complexity of the pathogenic events associated with the development of KS in HIV-infected individuals, HHV-8 is the etiologic agent of this disease. The initiation and/or propagation of KS requires an activated state and is mediated, at least in part, by cytokines. A number of factors, including TNF-α, IL-1β, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), basic fibroblast growth factor, and oncostatin M, function in an autocrine and paracrine manner to sustain the growth and chemotaxis of the KS spindle cells. In this regard, KSHV-derived IL-6 has been demonstrated to induce proliferation of lymphoma cells and to inhibit the cytostatic effects of IFN-α on KSHV-infected lymphoma cells.

IMMUNE RESPONSE TO HIV

As detailed above and below, following the initial burst of viremia during primary infection, HIV-infected individuals mount robust immune responses that in most cases substantially curtail the levels of plasma viremia and likely contribute to delaying the ultimate development of clinically apparent disease for a median of 10 years in untreated individuals. This immune response contains elements of both humoral and cell-mediated immunity involving both adaptive and innate immune responses (**Table 197-7**; **Fig. 197-27**). It is directed against multiple antigenic determinants of the HIV virion as well as against viral proteins expressed on the surface of infected cells. Ironically, those CD4+ T cells with T cell receptors specific for HIV are theoretically those CD4+ T cells most likely to be activated—and thus to serve as early targets for productive HIV infection and the cell death or dysfunction associated with infection. Thus, an early consequence of HIV infection is interference with and decrease of the helper T cell population needed to generate an effective immune response.

Although a great deal of investigation has been directed toward delineating and better understanding the components of this immune response, it remains unclear which immunologic effector mechanisms are most important in delaying progression of infection and which, if any, play a role in the pathogenesis of HIV disease. This lack of knowledge has also hampered the ability to develop an effective vaccine for HIV disease.

HUMORAL IMMUNE RESPONSE

Antibodies to HIV usually appear within 3–6 weeks and almost invariably within 12 weeks of primary infection (**Fig. 197-28**); rare exceptions are in individuals who have defects in the ability to produce HIV-specific antibodies. Detection of these antibodies forms the basis of most diagnostic screening tests for HIV infection. The appearance of HIV-binding antibodies detected by ELISA and Western blot assays occurs prior to the appearance of neutralizing antibodies; the latter generally appear following the initial decreases in plasma viremia and are more closely related to the appearance of HIV-specific

TABLE 197-7 Elements of the Immune Response to HIV

Humoral immunity
Binding antibodies
Neutralizing antibodies
Type specific
Group specific
Broadly neutralizing
Antibodies participating in antibody-dependent cellular cytotoxicity (ADCC)
Protective
Pathogenic (bystander killing)
Enhancing antibodies
Complement
Cell-mediated immunity
Helper CD4+ T lymphocytes
Class I MHC–restricted cytotoxic CD8+ T lymphocytes
CD8+ T cell–mediated inhibition (noncytolytic)
ADCC
Natural killer cells

Abbreviation: MHC, major histocompatibility complex.

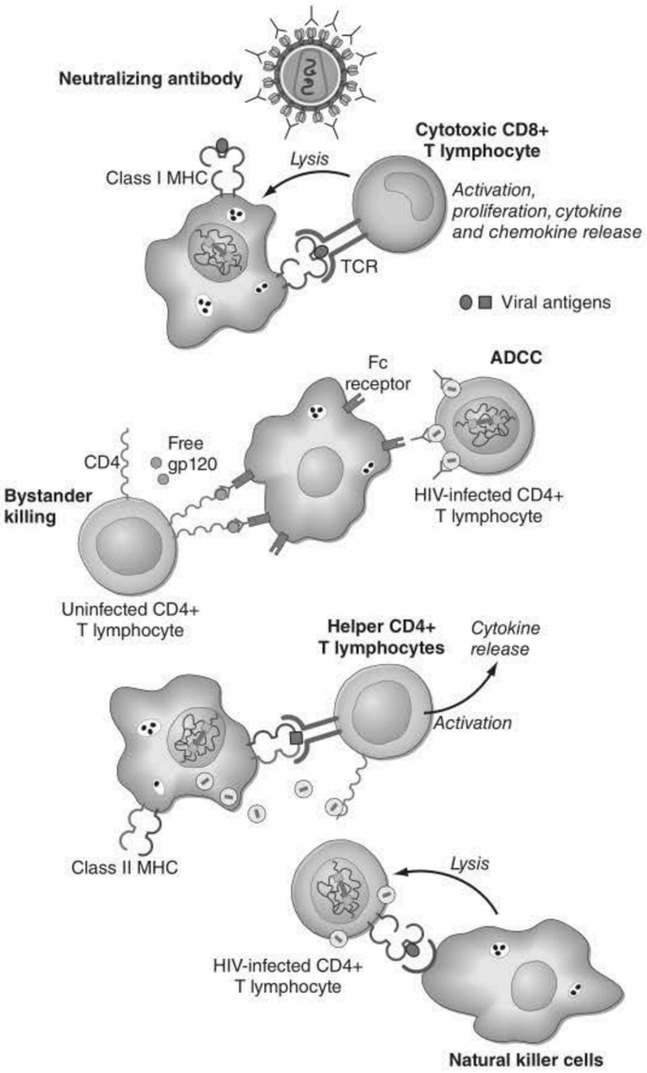

FIGURE 197-27 Schematic representation of the different immunologic effector mechanisms thought to be active in the setting of HIV infection. Detailed descriptions are given in the text. ADCC, antibody-dependent cellular cytotoxicity; MHC, major histocompatibility complex; TCR, T cell receptor.

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CD8+ T lymphocytes. The first antibodies detected are those directed against the immunodominant region of the envelope gp41, followed by the appearance of antibodies to the structural or gag protein p24 and the gag precursor p55. Antibodies to p24 gag are followed by the appearance of antibodies to the outer envelope glycoprotein (gp120), the gag protein p17, and the products of the *pol* gene (p31 and p66). In addition, one may see antibodies to the low-molecular-weight regulatory proteins encoded by the HIV genes *vpr*, *vpu*, *vif*, *rev*, *tat*, and *nef*. On rare occasion, levels of HIV-specific antibodies may decline during treatment of acute HIV infection.

While antibodies to multiple antigens of HIV are produced, the precise functional significance of these different antibodies is unclear. The only viral proteins that elicit neutralizing antibodies are the envelope proteins gp120 and gp41. Antibodies directed toward the envelope proteins of HIV have been characterized both as being protective and as possibly contributing to the pathogenesis of HIV disease. Among the protective antibodies are those that function to neutralize HIV directly and prevent the spread of infection to additional cells, as well as those that participate in ADCC. The first neutralizing antibodies are directed against the autologous infecting virus and appear after approximately 12 to 24 weeks of infection. Due to its high rate of mutation the virus is usually able to quickly escape these (and subsequent) neutralizing antibodies. One important mechanism of immune escape is the addition of N-linked glycosylation sites, forming a glycan shield that interferes with envelope recognition by these initial antibodies.

A number of broad and potent HIV-neutralizing envelope-specific antibodies have been isolated from HIV-infected individuals in studies designed to better understand the host response to HIV infection. Approximately 20% of patients develop antibodies capable of neutralizing highly diverse strains. These usually appear 2 or more years following infection in the face of continual viremia. These studies have revealed at least five major sites within the HIV envelope trimer that are able to elicit broadly neutralizing antibodies. These sites include antibodies directed toward the CD4 binding site (CD4bs) of gp120, those binding glycan-dependent epitopes in the V1/V2 region of gp120, those near the base of the V3 region of gp120, those binding to the gp120/gp41 bridge, and those binding to the membrane-proximal region of gp41 **(Fig. 197-29)**. Several of these antibodies contain unique features including high levels of somatic hypermutation, selective germline gene usage (especially for CD4bs antibodies), and long heavy chain complementary determining regions (especially CDRH3). Of note, while these antibodies are broadly neutralizing in vitro, their precise in vivo significance is unclear and the patients from whom they were derived demonstrate evidence of ongoing viral replication unless treated with cART.

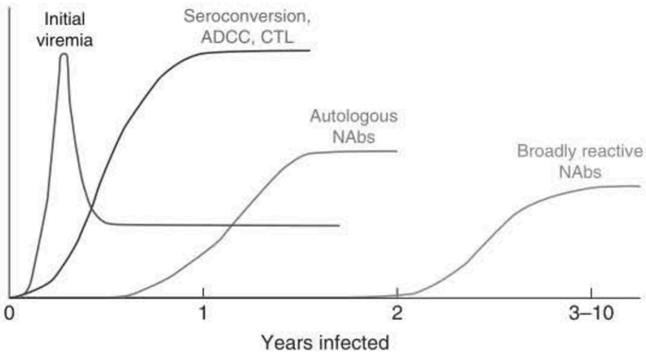

FIGURE 197-28 Relationship between initial HIV viremia and the development of antibodies to HIV. Within 3 to 6 weeks of initial HIV infection, non-neutralizing antibodies to HIV appear. These antibodies are capable of mediating antibody-dependent cellular cytotoxicity (ADCC). The decline in plasma viremia generally correlates with the appearance of cytotoxic T lymphocytes (CTL). After approximately 3 months, autologous neutralizing antibodies (NAbs) capable of neutralizing prior circulating strains of HIV appear. After 2 or more years, broadly reactive NAbs appear. *(Adapted from JT Mascola, DC Montefiori: Annu Rev Immunol 28:413, 2010.)*

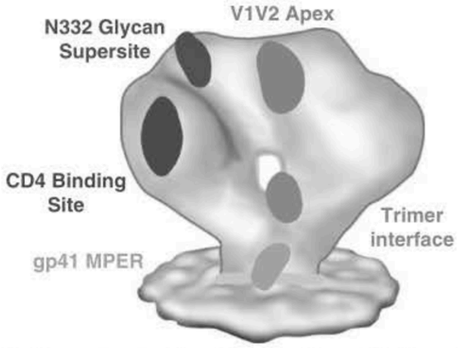

FIGURE 197-29 Known targets of broadly neutralizing antibodies against HIV-1. *(Adapted from PD Kwong, JR Mascola: Immunity 37:412, 2012.)*

The other major class of protective antibodies are those that participate in ADCC, a form of cell-mediated immunity **(Chap. 342)** in which NK cells that bear Fc receptors are armed with specific anti-HIV antibodies that bind to the NK cells via their Fc portion. These armed NK cells then bind to and destroy cells expressing HIV antigens. The levels of anti-envelope antibodies capable of mediating ADCC are highest in the earlier stages of HIV infection. Antibodies to both gp120 and gp41 have been shown to participate in ADCC-mediated killing of HIV-infected cells. In vitro, IL-2 can augment ADCC-mediated killing.

In addition to playing a role in host defense, HIV-specific antibodies have also been implicated in disease pathogenesis. Antibodies directed to gp41, when present in low titer, have been shown in vitro to be capable of facilitating infection of cells through an Fc receptor–mediated mechanism known as *antibody enhancement*. Thus, the same regions of the envelope protein of HIV that give rise to antibodies capable of mediating ADCC can also elicit the production of antibodies that can facilitate infection of cells in vitro. In addition, it has been postulated that anti-gp120 antibodies that participate in the ADCC killing of HIV-infected cells might also kill uninfected CD4+ T cells if the uninfected cells had bound free gp120, a phenomenon referred to as *bystander killing*.

One of the most primitive components of the humoral immune system is the complement system **(Chap. 342)**. This element of innate immunity consists of ~30 proteins that are found circulating in blood or associated with cell membranes. While HIV alone is capable of directly activating the complement cascade, the resulting lysis is weak due to the presence of host cell regulatory proteins captured in the virion envelope during budding. It is possible that complement-opsonized HIV virions have increased infectivity in a manner analogous to antibody-mediated enhancement.

CELLULAR IMMUNE RESPONSE

Given that T cell–mediated immunity is known to play a major role in host defense against most viral infections **(Chap. 342)**, it is generally thought to be an important component of the host immune response to HIV. T cell immunity can be divided into two major categories: that mediated by *helper/inducer CD4+ T cells* and that mediated by *cytotoxic/immunoregulatory CD8+ T cells*.

HIV-specific CD4+ T cells can be detected in the majority of HIV-infected patients through the use of flow cytometry to measure intracellular cytokine production in response to MHC class II tetramers pulsed with HIV peptides or through lymphocyte proliferation assays utilizing HIV antigens such as p24. These cells likely play a critical role in the orchestration of the immune response to HIV by providing help to HIV-specific B cells and CD8+ T cells. They may also be capable of directly killing HIV-infected cells. HIV-specific CD4+ T cells may be preferential targets of HIV infection by HIV-infected antigen-presenting cells during the generation of an immune response to HIV (Fig. 197-27). However, they also are likely to undergo clonal expansions in response to HIV antigens and thus survive as a population of cells. No clear correlations exist between levels of HIV-specific CD4+ T lymphocytes and plasma HIV RNA levels; however, in the setting

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of high viral loads, CD4+ T cell responses to HIV antigens appear to shift from one of proliferation and IL-2 production to one of IFN-γ production. Thus, while a reverse correlation exists between the level of p24-specific proliferation and levels of plasma HIV viremia, the nature of the causal relationship between these parameters is unclear.

MHC class I–restricted, HIV-specific CD8+ T cells have been identified in the peripheral blood of patients with HIV-1 infection. These cells include CTLs that produce perforins and T cells that can be induced by HIV antigens to express an array of cytokines such as IFN-γ, IL-2, MIP-1β, and TNF-α. CTLs have been identified in the peripheral blood of patients within weeks of HIV infection and prior to the appearance of plasma virus. The selective pressure they exert on the evolution of the population of circulating viruses reflects their potential role in control of HIV infection. These CD8+ T lymphocytes, through their HIV-specific antigen receptors, bind to and cause the lytic destruction of target cells bearing autologous MHC class I molecules presenting HIV antigens. Two types of CTL activity can be demonstrated in the peripheral blood or lymph node mononuclear cells of HIV-infected individuals. The first type directly lyses appropriate target cells in culture without prior in vitro stimulation (*spontaneous CTL activity*). The other type of CTL activity reflects the *precursor frequency of CTLs* (CTLp); this type of CTL activity can be demonstrated by stimulation of CD8+ T cells in vitro with a mitogen such as phytohemagglutinin or anti-CD3 antibody.

In addition to CTLs, CD8+ T cells capable of being induced by HIV antigens to express cytokines such as IFN-γ also appear in the setting of HIV-1 infection. It is not clear whether these are the same or different effector pools compared with those cells mediating cytotoxicity; in addition, the relative roles of each in host defense against HIV are not fully understood. It does appear that these CD8+ T cells are driven to in vivo expansion by HIV antigen. There is a direct correlation between levels of CD8+ T cells capable of producing IFN-γ in response to HIV antigens and plasma levels of HIV-1 RNA. Thus, while these cells are clearly induced by HIV-1 infection, their overall ability to control infection remains unclear. Multiple HIV antigens, including Gag, Env, Pol, Tat, Rev, and Nef, can elicit CD8+ T cell responses. Among patients who control viral replication in the absence of antiretroviral drugs are a subset of patients referred to as *elite nonprogressors* (see "Long-Term Survivors, Long-Term Nonprogressors, and Elite Controllers," above) whose peripheral blood contains a population of CD8+ T cells that undergo substantial in vitro proliferation and perforin expression in response to HIV antigens. It is possible that these cells play an important role in HIV-specific host defense.

At least three other forms of cell-mediated immunity to HIV have been described: non-cytolytic CD8+ T cell–mediated suppression of HIV replication, ADCC, and NK cell activity. *Non-cytolytic CD8+ T cell–mediated suppression of HIV replication* refers to the ability of CD8+ T cells from an HIV-infected patient to inhibit the replication of HIV in tissue culture without killing infected targets. There is no requirement for HLA compatibility between the CD8+ T cells and the HIV-infected cells. This effector mechanism is thus nonspecific and appears to be mediated by soluble factor(s) including the CC-chemokines RANTES (CCL5), MIP-1α (CCL3), and MIP-1β (CCL4). These CC-chemokines are potent suppressors of HIV replication and operate at least in part via blockade of the HIV co-receptor (*CCR5*) for R5 (macrophage-tropic) strains of HIV-1 (see above). *ADCC*, as described above in relation to humoral immunity, involves the killing of HIV-expressing cells by NK cells armed with specific antibodies directed against HIV antigens. Finally, *NK cells* alone have been shown to be capable of killing HIV-infected target cells in tissue culture. This primitive cytotoxic mechanism of host defense is directed toward nonspecific surveillance for neoplastic transformation and viral infection through recognition of altered class I MHC molecules.

DIAGNOSIS AND LABORATORY MONITORING OF HIV INFECTION

The establishment of HIV as the causative agent of AIDS and related syndromes early in 1984 was followed by the rapid development of sensitive screening tests for HIV infection. By March 1985, blood donors in the United States were routinely screened for antibodies to HIV. In 1996, blood banks in the United States added the p24 antigen capture assay to the screening process to help identify the rare infected individuals who were donating blood in the time (up to 3 months) between infection and the development of antibodies. In 2002, the ability to detect early infection with HIV was further enhanced by the licensure of nucleic acid testing (NAT) as a routine part of blood donor screening. These refinements decreased the interval between infection and detection (window period) from 22 days for antibody testing to 16 days with p24 antigen testing and subsequently to 12 days with NAT. The development of sensitive assays for monitoring levels of plasma viremia ushered in a new era of being able to monitor the progression of HIV disease more closely. Utilization of these tests, coupled with the measurement of levels of CD4+ T lymphocytes in peripheral blood, is essential in the management of patients with HIV infection.

DIAGNOSIS OF HIV INFECTION

The CDC has recommended that screening for HIV infection be performed as a matter of routine health care. The diagnosis of HIV infection depends on the demonstration of antibodies to HIV and/or the direct detection of HIV or one of its components. As noted above, antibodies to HIV generally appear in the circulation 3–12 weeks following infection.

The standard blood screening tests for HIV infection are based on the detection of antibodies to HIV. A common platform is the ELISA, also referred to as an *enzyme immunoassay* (EIA). This solid-phase assay is an extremely good screening test with a sensitivity of >99.5%. Most diagnostic laboratories use commercial kits that contain antigens from both HIV-1 and HIV-2 and thus are able to detect antibodies to either. These kits use both natural and recombinant antigens and are continuously updated to increase their sensitivity to newly discovered species, such as group O viruses (Fig. 197-1). The fourth-generation EIA tests combine detection of antibodies to HIV with detection of the p24 antigen of HIV. EIA tests are generally scored as positive (highly reactive), negative (nonreactive), or indeterminate (partially reactive). While the EIA is an extremely sensitive test, it is not optimal with regard to specificity. This is particularly true in studies of low-risk individuals, such as volunteer blood donors. In this latter population, only 10% of EIA-positive individuals are subsequently confirmed to have HIV infection. Among the factors associated with false-positive EIA tests are antibodies to class II antigens (such as may be seen following pregnancy, blood transfusion, or transplantation), autoantibodies, hepatic disease, recent influenza vaccination, and acute viral infections. For these reasons, anyone suspected of having HIV infection based on a positive or inconclusive fourth-generation EIA result should have the result confirmed with a more specific assay such as an HIV-1- or HIV-2-specific antibody immunoassay, a Western blot, or a plasma HIV RNA level. One can estimate whether an individual has a recent infection with HIV-1 by comparing the results on a standard EIA test that will score positive for all infected individuals with the results on an assay modified to be less sensitive ("detuned assay") that will score positive for individuals with established HIV infection and negative for individuals with recent infection. In rare instances, an HIV-infected individual treated early in the course of infection may revert to a negative EIA. This does *not* indicate clearing of infection; rather, it signifies levels of ongoing exposure to virus or viral proteins insufficient to maintain a measurable antibody response. When these individuals have discontinued therapy, viruses and antibodies have reappeared.

While current CDC recommendations indicate that a positive fourth-generation assay confirmed by a second HIV-1- or HIV-2-specific immunoassay is adequate for diagnosis, many feel it is prudent to confirm diagnosis with a second platform test such as the Western blot or HIV plasma RNA level. The Western blot **(Fig. 197-30)** assay takes advantage of the fact that multiple HIV antigens of different, well-characterized molecular weights elicit the production of specific antibodies. These antigens can be separated on the basis of molecular weight, and antibodies to each component can be detected as discrete bands on the Western blot. A negative Western blot is one in which no bands are present at molecular weights corresponding to HIV gene products. In a patient with a positive or indeterminate EIA

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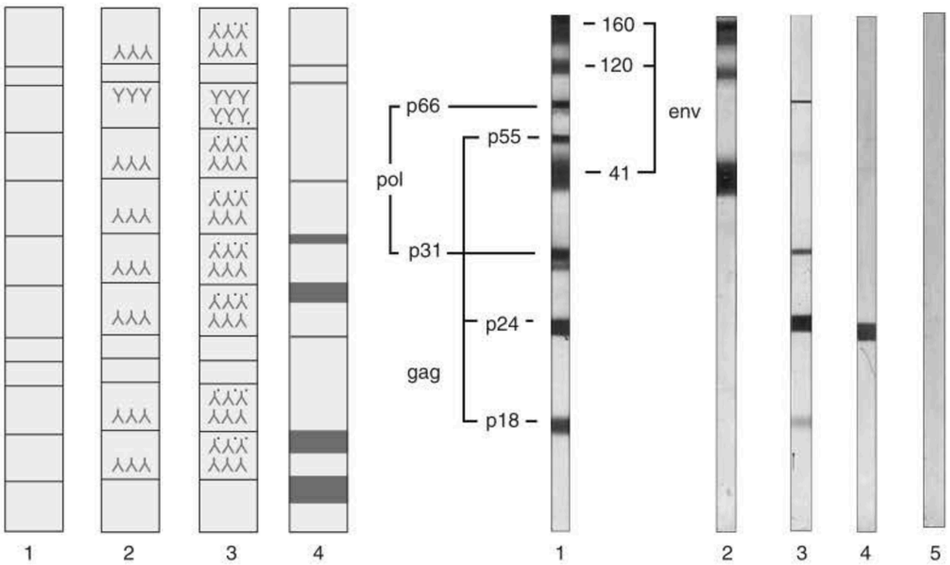

A
1. Virus digested: digest separated into components by molecular weight
2. Proteins transferred to filter paper: reaction with test serum
3. Enzyme-conjugated antihuman antibody added
4. Substrate added and color noted

B
1. Positive HIV-1 infection
2. gp 160 immunization
3. Indeterminate (HIV-2 infection)
4. Indeterminate (cross-reacting antibody to p24)
5. Negative

FIGURE 197-30 Western blot assay for detection of antibodies to HIV. *A.* Schematic representation of how a Western blot is performed. ***B.*** Examples of patterns of Western blot reactivity. In each instance the Western blot strip contains antigens to HIV-1. The serum from the patient immunized to the HIV-1 envelope gp160 contains only antibodies to the HIV-1 envelope proteins. The serum from the patient with HIV-2 infection cross-reacts with both *reverse transcriptase* and *gag* gene products of HIV-1.

and a negative Western blot, one can conclude with certainty that the EIA reactivity was a false positive. On the other hand, a Western blot demonstrating antibodies to products of all three of the major genes of HIV (*gag*, *pol*, and *env*) is conclusive evidence of infection with HIV. Criteria established by the FDA in 1993 state that a Western blot result is considered positive if antibodies exist to two of the three HIV proteins: p24, gp41, and gp120/160. Using these criteria, ~10% of all blood donors deemed positive for HIV-1 infection lacked an antibody band to the *pol* gene product p31. Some 50% of these blood donors were subsequently found to be false positives. Thus, the absence of the p31 band should increase the suspicion that one may be dealing with a false-positive test result. In this setting it is prudent to obtain additional confirmation with an RNA-based test for HIV-1 and/or a follow-up Western blot. By definition, Western blot patterns of reactivity that do not fall into the positive or negative categories are considered "indeterminate." There are two possible explanations for an indeterminate Western blot result. The most likely explanation in a low-risk individual is that the patient being tested has antibodies that cross-react with one of the proteins of HIV. The most common patterns of cross-reactivity are antibodies that react with p24 and/or p55. The least likely explanation in this setting is that the individual is infected with HIV and is in the process of mounting a classic antibody response. In either instance, the Western blot should be repeated in 1 month to determine whether the indeterminate pattern is a pattern in evolution. In addition, one may attempt to confirm a diagnosis of HIV infection with one of the tests for HIV RNA (discussed below). While the Western blot is an excellent confirmatory test for HIV infection in patients with a positive or indeterminate EIA, it is a poor screening test. Among individuals with a negative EIA and PCR for HIV, 20–30% may show one or more bands on Western blot. While these bands are usually faint and represent cross-reactivity, their presence creates a situation in which other diagnostic modalities (such as DNA PCR, RT-PCR, or p24 antigen capture) must be employed to ensure that the bands do not indicate early HIV infection.

A guideline for the use of these serologic tests in attempting to make a diagnosis of HIV infection is depicted in **Fig. 197-31**. In patients in whom HIV infection is suspected, the appropriate initial test is the EIA. If the result is negative, unless there is strong reason to suspect early HIV infection (as in a patient exposed within the previous 3 months), the diagnosis is ruled out and retesting should be performed only as clinically indicated. If the EIA is indeterminate or positive, the test should be repeated. If the repeat is negative on two occasions, one can assume that the initial positive reading was due to a technical error in the performance of the assay and that the patient is negative. If the repeat is indeterminate or positive, one should proceed to the HIV-1 Western blot. If the Western blot is positive, the diagnosis is HIV-1 infection. If the Western blot is negative, the EIA can be assumed to have been a false positive for HIV-1 and the diagnosis of HIV-1 infection is ruled out. It would also be prudent at this point to perform specific serologic testing for HIV-2 following the same type of algorithm. If the Western blot for HIV-1 is indeterminate, it should be repeated in 4–6 weeks; in addition, one may proceed to a specific HIV-1 or HIV-2 antibody differentiation assay, HIV-1 RNA assay, or HIV-1 DNA PCR. If the HIV RNA assays are negative and there is no progression in the Western blot, a diagnosis of HIV-1 is ruled out. If either HIV-1 RNA assay is positive and/or the HIV-1 Western blot shows progression, a tentative diagnosis of HIV-1 infection can be made and later confirmed with a follow-up Western blot demonstrating a positive pattern. In addition to these standard laboratory-based assays for detecting antibodies to HIV, a series of point-of-care tests can provide results in 1–60 min. Among the most popular of these is the OraQuick Rapid HIV-1 antibody test that can be run on blood, plasma, or saliva. The sensitivity and specificity of this test is ~99% when run on whole blood. Specificity remains the same but sensitivity drops to 98% when the test is run on saliva. While negative results from this test are adequate to rule out a diagnosis of HIV infection, a positive finding should be considered preliminary and confirmed with standard serologic testing, as described above. Two rapid test kits are licensed for home use. They are the OraQuick HIV test and the Home Access HIV-1 test system. A positive result with either of these tests should be followed with confirmatory testing by a healthcare professional.

A variety of laboratory tests are available for the direct detection of HIV or its components (**Table 197-8**). These tests may be of

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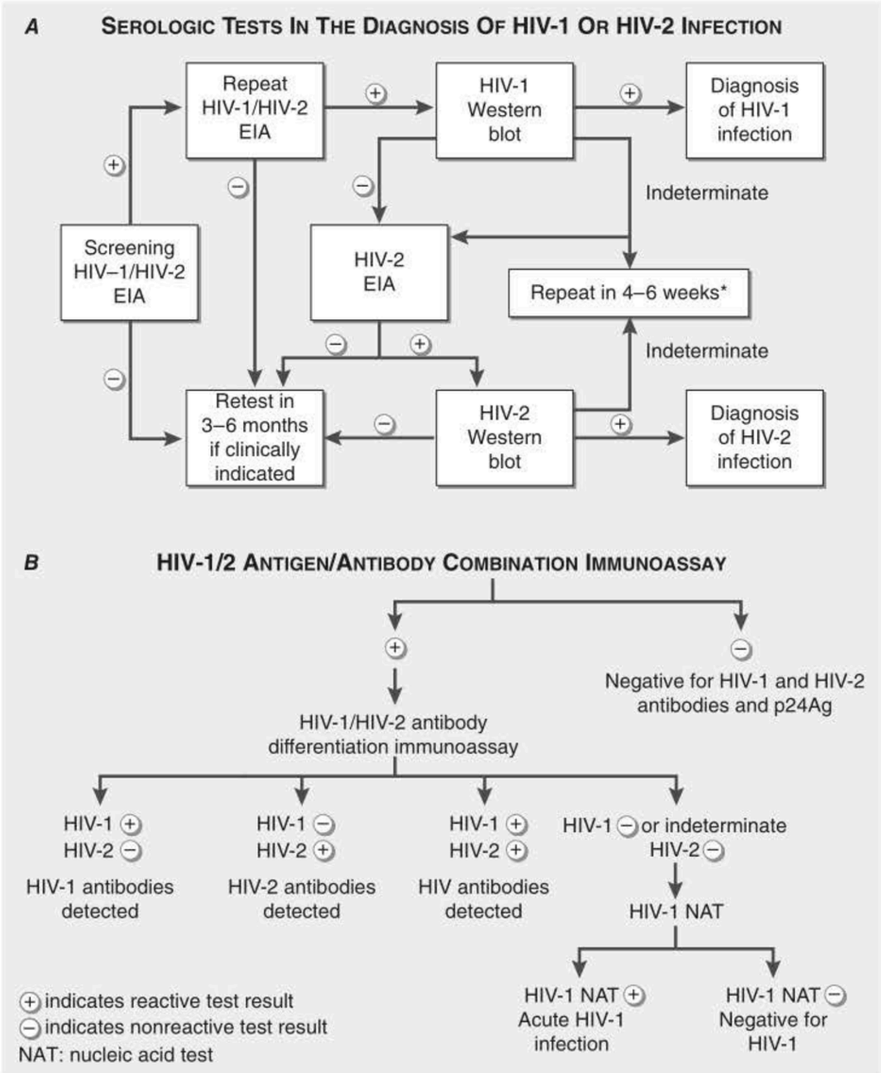

FIGURE 197-31 Serologic tests for the diagnosis of HIV-1 or HIV-2 infection. *A.* Algorithm including the use of a Western blot. *Stable indeterminate Western blot 4–6 weeks later makes HIV infection unlikely. However, it should be repeated twice at 3-month intervals to rule out HIV infection. Alternatively, one may test for HIV-1 p24 antigen or HIV RNA. EIA, enzyme immunoassay. ***B.*** CDC algorithm not including the use of a Western blot. *(Adapted from stacks.cdc.gov/view/cdc/23446.)*

considerable help in making a diagnosis of HIV infection when the antibody determination assays or Western blot results are indeterminate. In addition, the tests detecting levels of HIV RNA can be used to determine prognosis and to assess the response to antiretroviral therapies. The simplest, least expensive, and most rarely used of the direct detection tests is the *p24 antigen capture assay*. This is an EIA-type assay in which the solid phase consists of antibodies to the p24 antigen of HIV. It detects the viral protein p24 in the blood of HIV-infected individuals where it exists either as free antigen or complexed to anti-p24 antibodies. Overall, ~30% of individuals with untreated HIV infection have detectable levels of free p24 antigen. This increases to ~50% when samples are treated with a weak acid to dissociate antigen-antibody complexes. Throughout the course of HIV infection, an equilibrium exists between p24 antigen and anti-p24 antibodies. During the first

TABLE 197-8 Characteristics of Tests for Direct Detection of HIV

TEST	TECHNIQUE	SENSITIVITY[a]	COST/TEST[b]
Immune complex–dissociated p24 antigen capture assay	Measurement of levels of HIV-1 core protein in an EIA-based format following dissociation of antigen-antibody complexes by weak acid treatment	Positive in 50% of patients; detects down to 15 pg/mL of p24 protein	$1–2
HIV RNA by PCR	Target amplification of HIV-1 RNA via reverse transcription followed by PCR	Reliable to 40 copies/mL of HIV RNA	$75–150
HIV RNA by bDNA	Measurement of levels of particle-associated HIV RNA in a nucleic acid capture assay employing signal amplification	Reliable to 50 copies/mL of HIV RNA	$75–150
HIV RNA by TMA	Target amplification of HIV-1 RNA via reverse transcription followed by T7 RNA polymerase	Reliable to 100 copies/mL of HIV RNA	$225
HIV RNA by NASBA	Isothermal nucleic acid amplification with internal controls	Reliable to 80 copies/mL of HIV RNA	$75–150

[a]Sensitivity figures refer to those approved by the U.S. Food and Drug Administration. [b]Prices may be lower in large-volume settings.

Abbreviations: bDNA, branched DNA; cDNA, complementary DNA; EIA, enzyme immunoassay; NASBA, nucleic acid sequence–based amplification; PCR, polymerase chain reaction; TMA, transcription-mediated amplification.

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few weeks of infection, before an immune response develops, there is a brisk rise in p24 antigen levels. After the development of anti-p24 antibodies, these levels decline. Late in the course of infection, when circulating levels of virus are high, p24 antigen levels also increase, particularly when detected by techniques involving dissociation of antigen-antibody complexes. The p24 antigen capture assay has its greatest use as a screening test for HIV infection in patients suspected of having the acute HIV syndrome (see below), as high levels of p24 antigen are present prior to the development of antibodies. Its use as a stand-alone test for routine blood donor screening for HIV infection has been replaced by use of NAT or "fourth-generation" assays that combine antigen and antibody testing. The ability to measure and monitor levels of HIV RNA in the plasma of patients with HIV infection has been of extraordinary value in furthering our understanding of the pathogenesis of HIV infection, in monitoring the response to cART, and in providing a diagnostic tool in settings where measurements of anti-HIV antibodies may be misleading, such as in acute infection and neonatal infection. Four assays are predominantly used for this purpose. They are reverse transcriptase PCR (*RT-PCR*; Amplicor and RealTi*me*); branched DNA (*bDNA*; VERSANT); transcription-mediated amplification (*TMA*; APTIMA); and nucleic acid sequence–based amplification (*NASBA*; NucliSENS). These tests are of value in making a diagnosis of HIV infection, in establishing initial prognosis, and in monitoring the effects of therapy. In addition to the commercially available tests for measuring HIV RNA, *DNA PCR* assays are also employed by research laboratories for making a diagnosis of HIV infection by amplifying HIV proviral DNA from peripheral blood mononuclear cells. The commercially available RNA detection tests have a sensitivity of 40–80 copies of HIV RNA per milliliter of plasma. Research laboratory–based RNA assays can detect as few as one HIV RNA copy per milliliter, while the DNA PCR tests can detect proviral DNA at a frequency of one copy per 10,000–100,000 cells. Thus, these tests are extremely sensitive. One frequent consequence of a high degree of sensitivity is some loss of specificity, and false-positive results have been reported with each of these techniques. For this reason, a positive EIA with a confirmatory Western blot or HIV RNA assay remains the "gold standard" for a diagnosis of HIV infection, and the interpretation of other test results must be done with this in mind.

In the RT-PCR technique, following DNAse treatment, a cDNA copy is made of all RNA species present in plasma. Because HIV is an RNA virus, this will result in the production of DNA copies of the HIV genome in amounts proportional to the amount of HIV RNA present in plasma. This cDNA is then amplified and characterized using standard PCR techniques, employing primer pairs that can distinguish genomic cDNA from messenger cDNA. The bDNA assay involves the use of a solid-phase nucleic acid capture system and signal amplification through successive nucleic acid hybridizations to detect small quantities of HIV RNA. Both tests can achieve a tenfold increase in sensitivity to 40–50 copies of HIV RNA per milliliter with a preconcentration step in which plasma undergoes ultracentrifugation to pellet the viral particles. In the TMA assay, a cDNA copy of viral RNA is made using primers that contain a promoter sequence for T7 RNA polymerase. T7 polymerase is then added to produce multiple copies of RNA amplicon from the DNA template. It is qualified at 100 copies/mL. The NASBA technique involves the isothermal amplification of a sequence within the gag region of HIV in the presence of internal standards and employs the production of multiple RNA copies through the action of T7-RNA polymerase. The resulting RNA species are quantitated through hybridization with a molecular beacon DNA probe that is quenched in the absence of hybridization. The lower limit of detection for the NucliSENS assay is 80 copies/mL.

In addition to being a diagnostic and prognostic tool, RT-PCR and DNA-PCR are also useful for amplifying defined areas of the HIV genome for sequence analysis and have become an important technique for studies of sequence diversity and microbial resistance to antiretroviral agents. In patients with a positive or indeterminate EIA test and an indeterminate Western blot, and in patients in whom serologic testing may be unreliable (such as patients with hypogammaglobulinemia or advanced HIV disease), these tests for quantitating HIV RNA in plasma or detecting proviral DNA in peripheral blood mononuclear cells are valuable tools for making a diagnosis of HIV infection; however, they should be used for diagnosis only when standard serologic testing has failed to provide a definitive result.

LABORATORY MONITORING OF PATIENTS WITH HIV INFECTION

The epidemic of HIV infection and AIDS has provided the clinician with new challenges for integrating clinical and laboratory data to effect optimal patient management. The close relationship between clinical manifestations of HIV infection and CD4+ T cell count has made measurement of CD4+ T cell numbers a routine part of the evaluation of HIV-infected individuals. The discovery of HIV as the cause of AIDS led to the development of sensitive tests that allow one to monitor the levels of HIV in the blood. Determinations of peripheral blood CD4+ T cell counts and measurements of the plasma levels of HIV RNA provide a powerful set of tools for determining prognosis and monitoring response to therapy.

CD4+ T Cell Counts The CD4+ T cell count is the laboratory test generally accepted as the best indicator of the immediate state of immunologic competence of the patient with HIV infection. This measurement, which can be made directly or calculated as the product of the percentage of CD4+ T cells (determined by flow cytometry) and the total lymphocyte count (determined by the white blood cell count [WBC] multiplied by the lymphocyte differential percentage), has been shown to correlate very well with the level of immunologic competence. Patients with CD4+ T cell counts <200/μL are at high risk of disease from *P. jirovecii*, while patients with CD4+ T cell counts <50/μL are also at high risk of disease from CMV, mycobacteria of the *M. avium* complex (MAC), and/or *T. gondii* (**Fig. 197-32**). Once the CD4+ T cell count is <200/μL, patients should be placed on a regimen for *P. jirovecii* prophylaxis, and once the count is <50/μL, primary prophylaxis for MAC infection is indicated. As with any laboratory measurement, one may wish to obtain two determinations prior to any significant changes in patient management based on CD4+ T cell count alone. Patients with HIV infection should have CD4+ T cell measurements performed at the time of diagnosis and every 3–6 months thereafter. More frequent measurements should be made if a declining trend is noted. For patients who have been on cART for at least 2 years with HIV RNA levels persistently <50 copies/mL and CD4 counts >500/μl, the monitoring of the CD4 count is felt by many to be optional. There are a handful of clinical situations in which the CD4+ T cell count may be misleading. Patients with HTLV-1/HIV co-infection may have elevated CD4+ T cell counts that do not accurately reflect their degree of immune competence. In patients with hypersplenism or those who have undergone splenectomy, and in patients receiving medications that suppress the bone marrow such as IFN-α, the CD4+ T cell percentage may be a more reliable indication of immune function than the CD4+ T cell count. A CD4+ T cell percentage of 15 is comparable to a CD4+ T cell count of 200/μL.

HIV RNA Determinations Facilitated by highly sensitive techniques for the precise quantitation of small amounts of nucleic acids, the measurement of serum or plasma levels of HIV RNA has become an essential component in the monitoring of patients with HIV infection. As discussed in "Diagnosis of HIV Infection," above, the most commonly used technique is the RT-PCR assay. This assay generates data in the form of number of copies of HIV RNA per milliliter of serum or plasma and can reliably detect as few as 40 copies of HIV RNA per milliliter of plasma. Research-based assays can detect down to one copy per milliliter. While it is common practice to describe levels of HIV RNA below these cut-offs as "undetectable," this is a term that should be avoided as it is imprecise and leaves the false impression that the level of virus is 0. By utilizing more sensitive, nested PCR techniques and by studying tissue levels of virus as well as plasma levels, HIV RNA can be detected in virtually every patient with HIV infection. The one notable exception to this is a patient who underwent cytoreductive therapy followed by a bone marrow transplant from a CCR5Δ32 homozygous donor.

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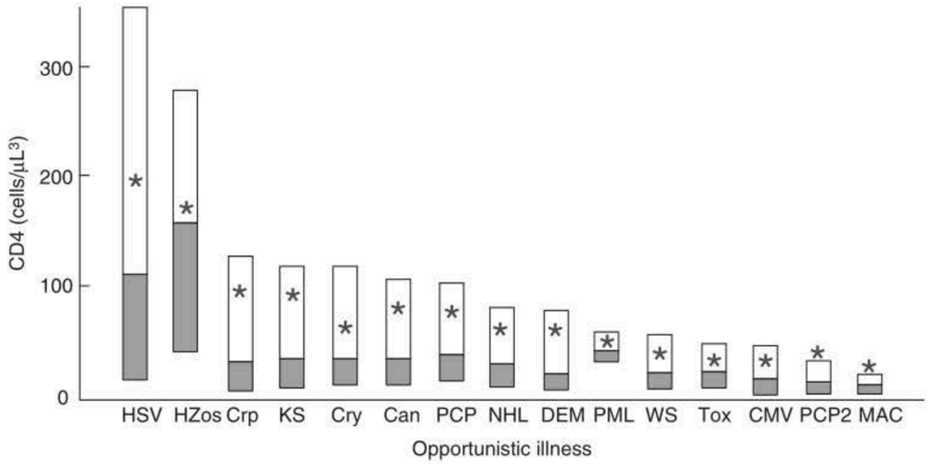

FIGURE 197-32 Relationship between CD4+ T cell counts and the development of opportunistic diseases. Boxplot of the median (line inside the box), first quartile (bottom of the box), third quartile (top of the box), and mean (asterisk) CD4+ lymphocyte count at the time of the development of opportunistic disease. Can, candidal esophagitis; CMV, cytomegalovirus infection; Crp, cryptosporidiosis; Cry, cryptococcal meningitis; DEM, AIDS dementia complex; HSV, herpes simplex virus infection; HZos, herpes zoster; KS, Kaposi's sarcoma; MAC, *Mycobacterium avium* complex bacteremia; NHL, non-Hodgkin's lymphoma; PCP, primary *Pneumocystis jirovecii* pneumonia; PCP2, secondary *P. jirovecii* pneumonia; PML, progressive multifocal leukoencephalopathy; Tox, *Toxoplasma gondii* encephalitis; WS, wasting syndrome. *(From RD Moore, RE Chaisson: Ann Intern Med 124:633, 1996.)*

Measurements of changes in HIV RNA levels over time have been of great value in delineating the relationship between levels of virus and rates of disease progression (Fig. 197-22), the rates of viral turnover, the relationship between immune system activation and viral replication, and the time to development of drug resistance. HIV RNA measurements are greatly influenced by the state of activation of the immune system and may fluctuate greatly in the setting of secondary infections or immunization. For these reasons, decisions based on HIV RNA levels should never be made on a single determination. Measurements of plasma HIV RNA levels should be made at the time of HIV diagnosis and every 3–6 months thereafter in the untreated patient. Following the initiation of therapy or any change in therapy, plasma HIV RNA levels should be monitored approximately every 4 weeks until the effectiveness of the therapeutic regimen is determined by the development of a new steady-state level of HIV RNA. In most instances of effective antiretroviral therapy the plasma level of HIV RNA will drop to <50 copies/mL within 6 months of the initiation of treatment. During therapy, levels of HIV RNA should be monitored every 3–6 months to evaluate the continuing effectiveness of therapy.

HIV Resistance Testing The availability of multiple antiretroviral drugs as treatment options has generated a great deal of interest in the potential for measuring the sensitivity of an individual's HIV viral quasispecies to different antiretroviral agents. HIV resistance testing can be done through either genotypic or phenotypic measurements. In the genotypic assays, sequence analyses of the HIV genomes obtained from patients are compared with sequences of viruses with known antiretroviral resistance profiles. In the phenotypic assays, the in vivo growth of viral isolates obtained from the patient is compared with the growth of reference strains of the virus in the presence or absence of different antiretroviral drugs. A modification of this phenotypic approach utilizes a comparison of the enzymatic activities of the reverse transcriptase, protease, or integrase genes obtained by molecular cloning of patients' isolates to the enzymatic activities of genes obtained from reference strains of HIV in the presence or absence of different drugs targeted to these genes. These tests are quite good in identifying those antiretroviral agents that have been utilized in the past and suggesting agents that may be of future value in a given patient. Resistance testing is recommended at the time of initial diagnosis and, if therapy is not initiated at that time, at the time of initiation of cART. Drug resistance testing is also indicated in the setting of virologic failure and should be performed while the patient is still on the failing regimen because of the propensity for the pool of HIV quasispecies to rapidly revert to wild-type in the absence of the selective pressures of cART. In the hands of experts, resistance testing enhances the short-term ability to decrease viral load by ~0.5 log compared with changing drugs merely on the basis of drug history. In addition to the use of resistance testing to help in the selection of new drugs in patients with virologic failure, it may also be of value in selecting an initial regimen for treatment of therapy-naïve individuals. This is particularly true in geographic areas with a high level of background resistance. The patient needs to have an HIV-1 RNA level above 500–1000 copies/mL for an accurate resistance determination. Resistance assays lose their consistency at lower levels of plasma viremia.

Co-Receptor Tropism Assays Following the licensure of maraviroc as the first CCR5 antagonist for the treatment of HIV infection (see below), it became necessary to be able to determine whether a patient's virus was likely to respond to this treatment. Patients tend to have CCR5-tropic virus early in the course of infection, with a trend toward CXCR4 viruses later in disease. The antiretroviral agent maraviroc is effective only against CCR5-tropic viruses. Because the genotypic determinants of cellular tropism are poorly defined, a phenotypic assay is necessary to determine this property of HIV. Two commercial assays, the Trofile assay (Monogram Biosciences) and the Phenoscript assay (VIRalliance), are available to make this determination. These assays clone the envelope regions of the patient's virus into an indicator virus that is then used to infect target cells expressing either CCR5 or CXCR4 as their co-receptor. These assays take weeks to perform and are expensive. Another, less costly option is to obtain a genotypic assay of the V3 region of HIV-1 and then employ a computer algorithm to predict viral tropism from the sequence. While this approach is less expensive than the classic phenotypic assay, there are fewer data to validate its predictive value.

Other Tests A variety of other laboratory tests have been studied as potential markers of HIV disease activity. Among these are quantitative culture of replication-competent HIV from plasma, peripheral blood mononuclear cells, or resting memory CD4+ T cells; circulating levels of β_2-microglobulin, soluble IL-2 receptor, IgA, acid-labile endogenous IFN, or TNF-α; and the presence or absence of activation markers such as CD38, HLA-DR, and PD-1 on CD4+ or CD8+ T cells. Nonspecific serologic markers of inflammation and/or coagulation such as IL-6, D-dimer, and sCD14 have been shown to have a high correlation with all-cause mortality (**Table 197-9**). While these measurements have value as markers of disease activity and help to increase our understanding of the pathogenesis of HIV disease, they do not currently play a major role in the monitoring of patients with HIV infection.

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TABLE 197-9 Association Between High-Sensitivity CRP, Il-6, and D-Dimer with All-Cause Mortality in Patients with HIV Infection

	UNADJUSTED		ADJUSTED	
MARKER	ODDS RATIO (FOURTH/FIRST)	*P*	ODDS RATIO (FOURTH/FIRST)	*P*
Hs-CRP	2.0	.05	2.8	.03
IL-6	8.3	<.0001	11.8	<.0001
D-dimer	12.4	<.0001	26.5	<.0001

Abbreviations: Hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin 6.

Source: From LH Kuller et al: PLoS Med 5:e203, 2008.

TABLE 197-10 Clinical Findings in the Acute HIV Syndrome

General	Neurologic
Fever	Meningitis
Pharyngitis	Encephalitis
Lymphadenopathy	Peripheral neuropathy
Headache/retroorbital pain	Myelopathy
Arthralgias/myalgias	Dermatologic
Lethargy/malaise	Erythematous maculopapular rash
Anorexia/weight loss	Mucocutaneous ulceration
Nausea/vomiting/diarrhea	

Source: From B Tindall, DA Cooper: AIDS 5:1, 1991.

CLINICAL MANIFESTATIONS

The clinical consequences of HIV infection encompass a spectrum ranging from an acute syndrome associated with primary infection to a prolonged asymptomatic state to advanced disease. It is best to regard HIV disease as beginning at the time of primary infection and progressing through various stages. As mentioned above, active virus replication and progressive immunologic impairment occur throughout the course of HIV infection in most patients. With the exception of the rare, true, "elite" virus controllers or long-term nonprogressors (see "Long-Term Survivors, Long-Term Nonprogressors, and Elite Controllers," above), HIV disease in untreated patients inexorably progresses even during the clinically latent stage. Since the mid-1990s, cART has had a major impact on preventing and reversing the progression of disease over extended periods of time in a substantial proportion of adequately treated patients. Today, a person diagnosed with HIV infection and treated with cART has a close to normal life expectancy.

ACUTE HIV INFECTION

It is estimated that 50–70% of individuals with HIV infection experience an acute clinical syndrome ~3–6 weeks after primary infection (**Fig. 197-33**). Varying degrees of clinical severity have been reported, and although it has been suggested that symptomatic seroconversion leading to the seeking of medical attention indicates an increased risk for an accelerated course of disease, there does not appear to be a correlation between the level of the initial burst of viremia in acute HIV infection and the subsequent course of disease. The typical clinical findings in the acute HIV syndrome are listed in **Table 197-10**; they occur along with a burst of plasma viremia. It has been reported that several symptoms of the acute HIV syndrome (fever, skin rash, pharyngitis, and myalgia) occur less frequently in those infected by injection drug use compared with those infected by sexual contact. The syndrome is typical of an acute viral syndrome and has been likened to acute infectious mononucleosis. Symptoms usually persist for one to several weeks and gradually subside as an immune response to HIV develops and the levels of plasma viremia decrease. Opportunistic infections have been reported during this stage of infection, reflecting the immunodeficiency that results from reduced numbers of CD4+ T cells and likely also from the dysfunction of CD4+ T cells owing to viral protein and endogenous cytokine-induced perturbations of cells (Table 197-5) associated with the extremely high levels of plasma viremia. The Fiebig staging system has been used to describe the different stages of acute HIV infection, ranging from Stage 1 (HIV RNA positive alone) to Stage VI (HIV RNA and full western blot positive). A number of immunologic abnormalities accompany the acute HIV syndrome, including multiphasic perturbations of the numbers of circulating lymphocyte subsets. The number of total lymphocytes and T cell subsets (CD4+ and CD8+) are initially reduced. An inversion of the CD4+/CD8+ T cell ratio occurs later because of a rise in the number of CD8+ T cells. In fact, there may be a selective and transient expansion of CD8+ T cell subsets, as determined by T cell receptor analysis (see above). The total circulating CD8+ T cell count may remain elevated or return to normal; however, CD4+ T cell levels usually remain somewhat depressed, although there may be a slight rebound toward normal. Lymphadenopathy occurs in ~70% of individuals with primary HIV infection. Most patients recover spontaneously from this syndrome and many are left with only a mildly depressed CD4+ T cell count that remains stable for a variable period before beginning its progressive decline; in some individuals, the CD4+ T cell count returns to the normal range. Approximately 10% of patients manifest a fulminant course of immunologic and clinical deterioration after primary infection, even after the disappearance of initial symptoms. In most patients, primary infection with or without the acute syndrome is followed by a prolonged period of clinical latency or smoldering low disease activity.

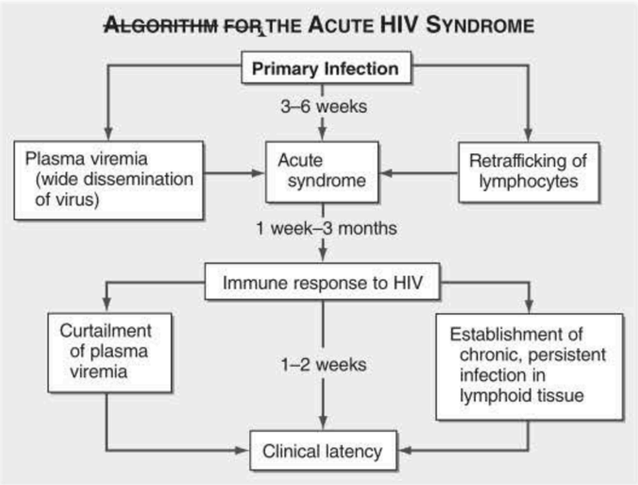

FIGURE 197-33 The acute HIV syndrome. See text for detailed description. *(Adapted from G Pantaleo et al: N Engl J Med 328:327, 1993. Copyright 1993 Massachusetts Medical Society. All rights reserved.)*

THE ASYMPTOMATIC STAGE—CLINICAL LATENCY

Although the length of time from initial infection to the development of clinical disease varies greatly, the median time for untreated patients is ~10 years. As emphasized above, HIV disease with active virus replication is ongoing and progressive during this asymptomatic period. The rate of disease progression is directly correlated with HIV RNA levels. Patients with high levels of HIV RNA in plasma progress to symptomatic disease faster than do patients with low levels of HIV RNA (Fig. 197-22). Some patients referred to as *long-term nonprogressors* show little if any decline in CD4+ T cell counts over extended periods of time. These patients generally have extremely low levels of HIV RNA; a subset, referred to as *elite nonprogressors*, exhibits HIV RNA levels <50 copies/mL. Certain other patients remain entirely asymptomatic despite the fact that their CD4+ T cell counts show a steady progressive decline to extremely low levels. In these patients, the appearance of an opportunistic disease may be the first manifestation of HIV infection. During the asymptomatic period of HIV infection, the average rate of CD4+ T cell decline is ~50/μL per year in an untreated patient. When the CD4+ T cell count falls to <200/μL, the resulting state of immunodeficiency is severe enough to place the patient at high risk for opportunistic infections and neoplasms and, hence, for clinically apparent disease.

SYMPTOMATIC DISEASE

Symptoms of HIV disease can appear at any time during the course of HIV infection. Generally speaking, the spectrum of illnesses that one observes changes as the CD4+ T cell count declines. The more severe

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and life-threatening complications of HIV infection occur in patients with CD4+ T cell counts <200/μL. A diagnosis of AIDS is made in any individual age 6 years and older with HIV infection and a CD4+ T cell count <200/μL (Stage 3, Table 197-2) and in anyone with HIV infection who develops one of the HIV-associated diseases considered to be indicative of a severe defect in cell-mediated immunity (Table 197-1). While the causative agents of the secondary infections are characteristically opportunistic organisms such as *P. jirovecii*, atypical mycobacteria, CMV, and other organisms that do not ordinarily cause disease in the absence of a compromised immune system, they also include several common bacterial and mycobacterial pathogens. Following the widespread use of cART and implementation of guidelines for the prevention of opportunistic infections **(Table 197-11)**, the incidence of these secondary infections has decreased dramatically **(Fig. 197-34)**. Overall, the clinical spectrum of HIV disease is constantly changing as patients live longer and new and better approaches to treatment and prophylaxis are developed. In addition to the classic AIDS-defining illnesses, patients with HIV infection also have an increase in several serious non-AIDS illnesses, including non-AIDS related cancers and cardiovascular, renal, and hepatic disease. Non-AIDS events dominate the disease burden for patients with HIV infection receiving cART (Table 197-4). In developed countries, AIDS-related illnesses are responsible for only ~25% of deaths in patients with HIV infection. A similar percentage of deaths are due to non-AIDS-defining malignancies, with cardiovascular disease and liver disease each accounting for approximately 15% of deaths. The physician providing care to a patient with HIV infection must be well versed in general internal medicine as well as HIV-related opportunistic diseases. In general, it should be stressed that a key element of treatment of symptomatic complications of HIV disease, whether they are primary or secondary, is achieving good control of HIV replication through the use of cART and instituting primary and secondary prophylaxis for opportunistic infections as indicated.

Diseases of the Respiratory System Acute bronchitis and sinusitis are prevalent during all stages of HIV infection. The most severe cases tend to occur in patients with lower CD4+ T cell counts. Sinusitis presents as fever, nasal congestion, and headache. The diagnosis is made by CT or MRI. The maxillary sinuses are most commonly involved; however, disease is also frequently seen in the ethmoid, sphenoid, and frontal sinuses. While some patients may improve without antibiotic therapy, radiographic improvement is quicker and more pronounced in patients who have received antimicrobial therapy. It is postulated that this high incidence of sinusitis results from an increased frequency of infection with encapsulated organisms such as *H. influenzae* and *Streptococcus pneumoniae*. In patients with low CD4+ T cell counts one may see mucormycosis infections of the sinuses. In contrast to the course of this infection in other patient populations, mucormycosis of the sinuses in patients with HIV infection may progress more slowly. In this setting aggressive, frequent local debridement in addition to local and systemic amphotericin B may result in effective treatment.

Pulmonary disease is one of the most frequent complications of HIV infection. The most common manifestation of pulmonary disease is pneumonia. Three of the 10 most common AIDS-defining illnesses are recurrent bacterial pneumonia, tuberculosis, and pneumonia due to the unicellular fungus *P. jirovecii*. Other major causes of pulmonary infiltrates include other mycobacterial infections, other fungal infections, nonspecific interstitial pneumonitis, KS, and lymphoma.

Bacterial pneumonia is seen with an increased frequency in patients with HIV infection, with 0.8–2.0 cases per 100 person-years. Patients with HIV infection are particularly prone to infections with encapsulated organisms. *S. pneumoniae* **(Chap. 141)** and *H. influenzae* **(Chap. 152)** are responsible for most cases of bacterial pneumonia in patients with AIDS. This may be a consequence of altered B cell function and/or defects in neutrophil function that may be secondary to HIV disease (see above). Pneumonias due to *S. aureus* **(Chap. 142)** and *P. aeruginosa* **(Chap. 159)** also are reported to occur with an increased frequency in patients with HIV infection. *S. pneumoniae* (pneumococcal) infection may be the earliest serious infection to occur in patients with HIV disease. This can present as pneumonia, sinusitis, and/or bacteremia. Patients with untreated HIV infection have a sixfold increase in the incidence of pneumococcal pneumonia and a 100-fold increase in the incidence of pneumococcal bacteremia. Pneumococcal disease may be seen in patients with relatively intact immune systems. In one study, the baseline CD4+ T cell count at the time of a first episode of pneumococcal pneumonia was ~300/μL. Of interest is the fact that the inflammatory response to pneumococcal infection appears proportional to the CD4+ T cell count. Due to this high risk of pneumococcal disease, immunization with the conjugated pneumococcal vaccine followed by booster immunization with the 23-valent pneumococcal polysaccharide vaccine is one of the generally recommended prophylactic measures for patients with HIV infection. This is likely most effective if given while the CD4+ T cell count is >200/μL and, if given to patients with lower CD4+ T cell counts, should be repeated once the count has been above 200 for 6 months. Although clear guidelines do not exist, it also makes sense to repeat immunization every 5 years. The incidence of bacterial pneumonia is cut in half when patients quit smoking.

Pneumocystis pneumonia (PCP), once the hallmark of AIDS, has dramatically declined in incidence following the development of effective prophylactic regimens and the widespread use of cART. It is, however, still the single most common cause of pneumonia in patients with HIV infection in the United States and can be identified as a likely etiologic agent in 25% of cases of pneumonia in patients with HIV infection, with an incidence in the range of 2–3 cases per 100 person-years. Approximately 30% of cases of HIV-associated PCP occur in patients who are unaware of their HIV status. The risk of PCP is greatest among those who have experienced a previous bout of PCP and those who have CD4+ T cell counts of <200/μL. Overall, 79% of patients with PCP have CD4+ T cell counts <100/μL and 95% of patients have CD4+ T cell counts <200/μL. Recurrent fever, night sweats, thrush, and unexplained weight loss also are associated with an increased incidence of PCP. For these reasons, it is strongly recommended that all patients with CD4+ T cell counts <200/μL (or a CD4 percentage <15) receive some form of PCP prophylaxis. The incidence of PCP is approaching zero in patients with known HIV infection receiving appropriate cART and prophylaxis. In the United States, primary PCP is now occurring at a median CD4+ T cell count of 36/μL, while secondary PCP is occurring at a median CD4+ T cell count of 10/μL. Patients with PCP generally present with fever and a cough that is usually nonproductive or productive of only scant amounts of white sputum. They may complain of a characteristic retrosternal chest pain that is worse on inspiration and is described as sharp or burning. HIV-associated PCP may have an indolent course characterized by weeks of vague symptoms and should be included in the differential diagnosis of fever, pulmonary complaints, or unexplained weight loss in any patient with HIV infection and <200 CD4+ T cells/μL. The most common finding on chest x-ray is either a normal film, if the disease is suspected early, or a faint bilateral interstitial infiltrate. The classic finding of a dense perihilar infiltrate is unusual in patients with AIDS. In patients with PCP who have been receiving aerosolized pentamidine for prophylaxis, one may see an x-ray picture of upper lobe cavitary disease, reminiscent of TB. Other less common findings on chest x-ray include lobar infiltrates and pleural effusions. Thin-section CT may demonstrate a patchy ground-glass appearance. Routine laboratory evaluation is usually of little help in the differential diagnosis of PCP. A mild leukocytosis is common, although this may not be obvious in patients with prior neutropenia. Elevation of lactate dehydrogenase is common. Arterial blood-gases may indicate hypoxemia with a decline in Pao_2 and an increase in the arterial-alveolar (a–A) gradient. Arterial blood-gas measurements not only aid in making the diagnosis of PCP but also provide important information for staging the severity of the disease and directing treatment (see below). A definitive diagnosis of PCP requires demonstration of the organism in samples obtained from induced sputum, bronchoalveolar lavage, transbronchial biopsy, or open-lung biopsy. PCR has been used to detect specific DNA sequences for *P. jirovecii* in clinical specimens where histologic examinations have failed to make a diagnosis.

In addition to pneumonia, a number of other clinical problems have been reported in HIV-infected patients as a result of infection

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TABLE 197-11 NIH/CDC/IDSA 2013 Guidelines for the Prevention of Opportunistic Infections in Persons Infected with HIV

PATHOGEN	INDICATIONS	FIRST CHOICE(S)	ALTERNATIVES
Recommended as Standard of Care for Primary and Secondary Prophylaxis			
Pneumocystis jirovecii	CD4+ T cell count <200/μL *or* Oropharyngeal candidiasis *or* Prior bout of PCP	Trimethoprim-sulfamethoxazole (TMP-SMX), 1 DS tablet qd PO *or* TMP-SMX, 1 SS tablet qd PO	Dapsone 50 mg bid PO or 100 mg/d PO *or* Dapsone 50 mg/d PO + Pyrimethamine 50 mg/week PO + Leucovorin 25 mg/week PO *or* (Dapsone 200 mg PO + Pyrimethamine 75 mg PO + Leucovorin 25 mg) weekly PO *or* Aerosolized pentamidine, 300 mg via Respirgard II nebulizer every month *or* Atovaquone 1500 mg/d PO *or* TMP-SMX 1 DS tablet 3×/week PO
	May stop prophylaxis if CD4+ T cell count >200/μL for ≥3 months		
Mycobacterium tuberculosis Isoniazid sensitive	Skin test >5 mm *or* Positive IFN-γ release assay *or* Prior positive test without treatment *or* Close contact with case of active pulmonary TB Same with high probability of exposure to drug-resistant TB	(Isoniazid 300 mg PO + Pyridoxine 25 mg PO) qd × 9 months *or* Isoniazid 900 mg PO twice weekly + Pyridoxine 25 mg PO daily × 9 months	Rifabutin (dose adjusted based on cART regimen) or rifampin 600 mg PO qd × 4 months
Drug resistant	Consult local public health authorities		
Mycobacterium-avium complex	CD4+ T cell count <50/μL	Azithromycin 1200 mg weekly PO or 600 mg twice weekly PO *or* Clarithromycin 500 mg bid PO	Rifabutin (dose adjusted based on cART regimen)
	Prior documented disseminated disease	Clarithromycin 500 mg bid PO + Ethambutol 15 (mg/kg)/d PO	Azithromycin 500–600 mg/d PO + Ethambutol 15 (mg/kg)/d PO
	May stop prophylaxis if CD4+ T cell count >100/μL for ≥6 months		
Toxoplasma gondii	TOXO IgG antibody positive and CD4+ T cell count <100/μL	TMP-SMX 1 DS tablet PO qd	TMP-SMX 1 DS 3× weekly PO *or* TMP-SMX, 1 SS PO daily *or* Dapsone 50 mg/d PO + Pyrimethamine 50 mg weekly PO + Leucovorin 25 mg weekly PO *or* (Dapsone 200 mg PO + Pyrimethamine 75 mg PO + Leucovorin 25 mg PO) weekly *or* Atovaquone 1500 mg PO daily ± (Pyrimethamine 25 mg PO + Leucovorin 10 mg PO) daily
	Prior toxoplasmic encephalitis and CD4+ T cell count <200/μL	Sulfadiazine 2000–4000 mg in 2–4 divided doses daily PO + Pyrimethamine 25–50 mg/d PO + Leucovorin 10–25 mg/d PO	Clindamycin 600 mg q8h PO + Pyrimethamine 25–50 mg/d PO + Leucovorin 10–25 mg/d PO *or* TMP-SMX 1 DS tablet bid *or* Atovaquone 750–1500 mg PO bid ± (Pyrimethamine 25 mg/d PO + Leucovorin 10 mg/d PO) or Sulfadiazine 2000–4000 mg/d (in 2–4 divided doses) PO

(*Continued*)

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TABLE 197-11 NIH/CDC/IDSA 2013 Guidelines for the Prevention of Opportunistic Infections in Persons Infected with HIV (Continued)

PATHOGEN	INDICATIONS	FIRST CHOICE(S)	ALTERNATIVES
Toxoplasma gondii (Continued)	May stop prophylaxis if CD4+ T cell count >200/μL for ≥3 months		
Varicella zoster virus	Significant exposure to chickenpox or shingles in a patient with no history of immunization or prior exposure to either	Varicella zoster immune globulin, IM, within 10 d of exposure (800-843-7477)	Acyclovir 800 mg PO 5 × day for 5–7 days *or* Valacyclovir 1 g PO tid for 5–7 days
Cryptococcus neoformans	Prior documented disease	Fluconazole 200 mg/d PO	Itraconazole 200 mg/d PO
	May stop prophylaxis if CD4+ T cell count >100/μL, no evidence of active fungal infection, and HIV RNA levels <500 copies/mL for >3 months		
Histoplasma capsulatum	Prior documented disease or CD4+ T cell count <150μL and high risk (endemic area or occupational exposure)	Itraconazole 200 mg bid PO	Fluconazole 400 mg/d PO
	May stop prophylaxis after 1 year if CD4+ T cell count >150/μL and patient on cART for ≥6 months		
Coccidioides immitis	Prior documented disease *or* positive serology and CD4+ T cell count <250/μL if from a disease endemic area. (For this indication prophylaxis can be stopped if CD4+ T cell count ≥250 for 6 months.)	Fluconazole 400 mg/d PO	
Penicillium marneffei	Prior documented disease Patients with CD4+T cell counts <100 who live or stay in northern Thailand, Southern China, or Vietnam	Itraconazole 200 mg/d PO	Fluconazole 400 mg PO once weekly
	May stop secondary prophylaxis in patients on ARV therapy with CD4+ T cell count >100/μL for ≥6 months		
Salmonella species	Prior recurrent bacteremia	Ciprofloxacin 500 mg bid PO for ≥6 months	
Bartonella	Prior infection	Doxycycline 200 mg/d PO *or* Azithromycin 1200 mg weekly PO *or* Clarithromycin 500 mg bid PO	
	May stop if CD4+ T cell count >200/μL for >3 months		
Cytomegalovirus	Prior end-organ disease	Valganciclovir 900 mg bid PO	Cidofovir 5 mg/kg every other week IV + Probenecid *or* Foscarnet 90–120 (mg/kg)/d IV
	May stop prophylaxis if CD4+ T cell count >100/μL for 6 months and no evidence of active CMV disease Restart if prior retinitis and CD4+ T cells <100/μL		
Immunizations Generally Recommended			
Hepatitis B virus	All susceptible (anti-HBc- and anti-HBs-negative) patients	Hepatitis B vaccine: 3 doses	
Hepatitis A virus	All susceptible (anti-HAV-negative) patients	Hepatitis A vaccine: 2 doses	
Influenza virus	All patients annually	Inactivated trivalent influenza virus vaccine 1 dose yearly	Oseltamivir 75 mg PO qd *or* Rimantadine or amantadine 100 mg PO bid (influenza A only)
Streptococcus pneumoniae	All patients, preferably before CD4+ T cell count ≤200/μL	Pneumococcal conjugated vaccine (13) 0.5 mL IM × 1 followed in 8 weeks or more by pneumococcal polysaccharide vaccine (23) if CD4+ T cell count >200/μL	
	~~Patients initially immunized at a CD4+ T cell count <100/μL~~ Patients initially immunized at a CD4+ T cell count <100/μL whose CD4+ T cell count then increases to>200/μL	Reimmunize	
Human papillomavirus	All patients 13–26 years of age	HPV vaccine; 3 doses	

(*Continued*)

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TABLE 197-11 NIH/CDC/IDSA 2013 Guidelines for the Prevention of Opportunistic Infections in Persons Infected with HIV (Continued)

PATHOGEN	INDICATIONS	FIRST CHOICE(S)	ALTERNATIVES
Recommended for Prevention of Severe or Frequent Recurrences			
Herpes simplex	Frequent/severe recurrences	Valacyclovir 500 mg bid PO *or* Acyclovir 400 mg bid PO *or* Famciclovir 500 mg bid PO	
Candida	Frequent/severe recurrences	Fluconazole 100–200 mg/d PO	Posaconazole 400 mg bid PO

Abbreviations: ARV, antiretroviral; bid, twice daily; DS, double-strength; PCP, *Pneumocystis jirovecii* pneumonia; PO, by mouth; SS, single-strength; TB, tuberculosis.

with *P. jirovecii*. Otic involvement may be seen as a primary infection, presenting as a polypoid mass involving the external auditory canal. In patients receiving aerosolized pentamidine for prophylaxis against PCP, one may see a variety of extrapulmonary manifestations of *P. jirovecii*. These include ophthalmic lesions of the choroid, a necrotizing vasculitis that resembles Buerger disease, bone marrow hypoplasia, and intestinal obstruction. Other organs that have been involved include lymph nodes, spleen, liver, kidney, pancreas, pericardium, heart, thyroid, and adrenals. Organ infection may be associated with cystic lesions that may appear calcified on CT or ultrasound.

The standard treatment for PCP or disseminated pneumocystosis is trimethoprim-sulfamethoxazole (TMP-SMX). A high (20–85%) incidence of side effects, particularly skin rash and bone marrow suppression, is seen with TMP-SMX in patients with HIV infection. Alternative treatments for mild to moderate PCP include dapsone/trimethoprim, clindamycin/primaquine, and atovaquone. IV pentamidine is the treatment of choice for severe disease in the patient unable to tolerate TMP-SMX. For patients with a Pa_{o2} <70 mmHg or with an a–A gradient

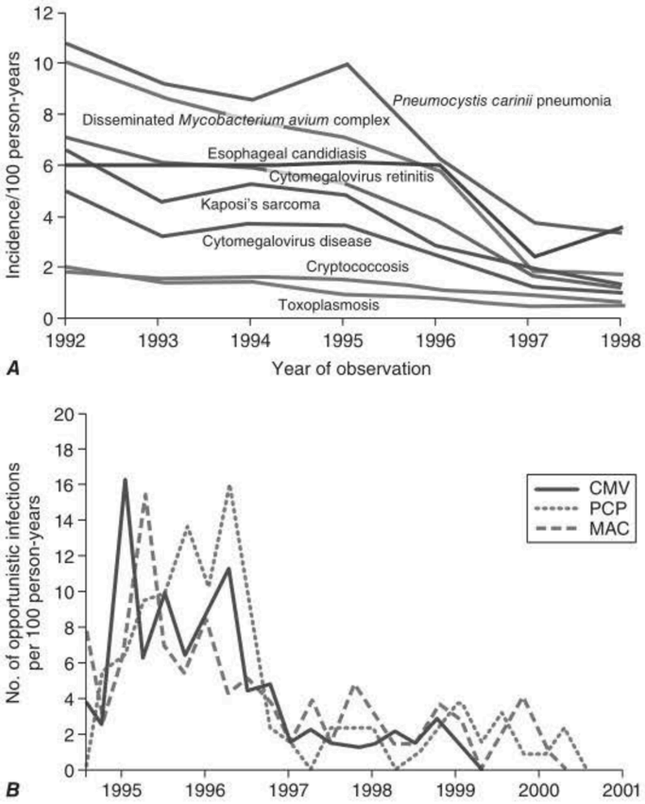

FIGURE 197-34 ***A.*** Decrease in the incidence of opportunistic infections and Kaposi's sarcoma in HIV-infected individuals with CD4+ T cell counts <100/μL from 1992 through 1998. *(Adapted and updated from FJ Palella et al: N Engl J Med 338:853, 1998, and JE Kaplan et al: Clin Infect Dis 30[S1]:S5, 2000, with permission.)* ***B.*** Quarterly incidence rates of cytomegalovirus (CMV), *Pneumocystis jirovecii* pneumonia (PCP), and *Mycobacterium avium* complex (MAC) from 1995 to 2001. *(From FJ Palella et al: AIDS 16:1617, 2002.)*

>35 mmHg, adjunct glucocorticoid therapy should be used in addition to specific antimicrobials. Overall, treatment should be continued for 21 days and followed by secondary prophylaxis. Prophylaxis for PCP is indicated for any HIV-infected individual who has experienced a prior bout of PCP, any patient with a CD4+ T cell count of <200/μL or a CD4 percentage <15, any patient with unexplained fever for >2 weeks, and any patient with a recent history of oropharyngeal candidiasis. The preferred regimen for prophylaxis is TMP-SMX, one double-strength tablet daily. This regimen also provides protection against toxoplasmosis and some bacterial respiratory pathogens. For patients who cannot tolerate TMP-SMX, alternatives for prophylaxis include dapsone plus pyrimethamine plus leucovorin, aerosolized pentamidine administered by the Respirgard II nebulizer, and atovaquone. Primary or secondary prophylaxis for PCP can be discontinued in those patients treated with cART who maintain good suppression of HIV (<50 copies/mL) and CD4+ T cell counts >200/μL for at least 3 months.

M. tuberculosis, once thought to be on its way to extinction in the United States, experienced a resurgence associated with the HIV epidemic (Chap. 173). Worldwide, approximately one-third of all AIDS-related deaths are associated with TB, and TB is the primary cause of death for 10–15% of patients with HIV infection. In the United States ~5% of AIDS patients have active TB. Patients with HIV infection are more likely to have active TB by a factor of 100 when compared with an HIV-negative population. For an asymptomatic HIV-negative person with a positive purified protein derivative (PPD) skin test, the risk of reactivation TB is around 1% per year. For the patient with untreated HIV infection, a positive PPD skin test, and no signs or symptoms of TB, the rate of reactivation TB is 7–10% per year. Untreated TB can accelerate the course of HIV infection. Levels of plasma HIV RNA increase in the setting of active TB and decline in the setting of successful TB treatment. Active TB is most common in patients 25–44 years of age, in African Americans and Hispanics, in patients in New York City and Miami, and in patients in developing countries. In these demographic groups, 20–70% of the new cases of active TB are in patients with HIV infection. The epidemic of TB embedded in the epidemic of HIV infection probably represents the greatest health risk to the general public and the health care profession associated with the HIV epidemic. In contrast to infection with atypical mycobacteria such as MAC, active TB often develops relatively early in the course of HIV infection and may be an early clinical sign of HIV disease. In one study, the median CD4+ T cell count at presentation of TB was 326/μL. The clinical manifestations of TB in HIV-infected patients are quite varied and generally show different patterns as a function of the CD4+ T cell count. In patients with relatively high CD4+ T cell counts, the typical pattern of pulmonary reactivation occurs: patients present with fever, cough, dyspnea on exertion, weight loss, night sweats, and a chest x-ray revealing cavitary apical disease of the upper lobes. In patients with lower CD4+ T cell counts, disseminated disease is more common. In these patients the chest x-ray may reveal diffuse or lower-lobe bilateral reticulonodular infiltrates consistent with miliary spread, pleural effusions, and hilar and/or mediastinal adenopathy. Infection may be present in bone, brain, meninges, GI tract, lymph nodes (particularly cervical lymph nodes), and viscera. Some patients with advanced HIV infection and active TB may have no symptoms of illness, and thus screening for TB should be part of the initial evaluation of every patient with HIV infection. Approximately 60–80% of HIV-infected patients

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with TB have pulmonary disease, and 30–40% have extrapulmonary disease. Respiratory isolation and a negative-pressure room should be used for patients in whom a diagnosis of pulmonary TB is being considered. This approach is critical to limit nosocomial and community spread of infection. Culture of the organism from an involved site provides a definitive diagnosis. Blood cultures are positive in 15% of patients. This figure is higher in patients with lower CD4 +T cell counts. In the setting of fulminant disease one cannot rely on the accuracy of a negative PPD skin test to rule out a diagnosis of TB. In addition, IFN-γ release assays may be difficult to interpret due to high backgrounds as a consequence of HIV-associated immune activation. TB is one of the conditions associated with HIV infection for which cure is possible with appropriate therapy. Therapy for TB is generally the same in the HIV-infected patient as in the HIV-negative patient **(Chap. 173)**. Due to the possibility of multidrug-resistant or extensively drug-resistant TB, drug susceptibility testing should be performed to guide therapy. Due to pharmacokinetic interactions, adjusted doses of rifabutin and/or changes in cART are required when treating TB in the setting of HIV infection. Treatment is most effective in programs that involve directly observed therapy. Initiation of cART and/or anti-TB therapy may be associated with clinical deterioration due to immune reconstitution inflammatory syndrome (IRIS) reactions. These are most common in patients initiating both treatments at the same time, may occur as early as 1 week after initiation of cART therapy, and are seen more frequently in patients with advanced HIV disease. For these reasons it is recommended that initiation of cART be delayed in antiretroviral-naïve patients with CD4 counts >50 cells/μL until 2–4 weeks following the initiation of treatment for TB. For patients with lower CD4 counts the benefits of more immediate cART outweigh the risks of IRIS, and cART should be started as soon as possible in those patients. Effective prevention of active TB can be a reality if the health care professional is aggressive in looking for evidence of latent or active TB by making sure that all patients with HIV infection receive a PPD skin test or evaluation with an IFN-γ release assay. Anergy testing is not of value in this setting. Since these tests rely on the host mounting an immune response to *M. tuberculosis*, patients with CD4+ T cell counts <200 cells/μL should be retested if their CD4+ T cell counts rise to persistently above 200. Patients at risk of continued exposure to TB should be tested annually. HIV-infected individuals with a skin-test reaction of >5 mm, those with a positive IFN-γ release assay, or those who are close household contacts of persons with active TB should receive treatment with 9 months of isoniazid and pyridoxine.

Atypical mycobacterial infections are also seen with an increased frequency in patients with HIV infection. Infections with at least 12 different mycobacteria have been reported, including *M. bovis* and representatives of all four Runyon groups. The most common atypical mycobacterial infection is with *M. avium* or *M. intracellulare* species—the *Mycobacterium avium* complex (MAC). Infections with MAC are seen mainly in patients in the United States and are rare in Africa. It has been suggested that prior infection with *M. tuberculosis* decreases the risk of MAC infection. MAC infections probably arise from organisms that are ubiquitous in the environment, including both soil and water. There is little evidence for person-to-person transmission of MAC infection. The presumed portals of entry are the respiratory and GI tracts. MAC infection is a late complication of HIV infection, occurring predominantly in patients with CD4+ T cell counts of <50/μL. The average CD4+ T cell count at the time of diagnosis is 10/μL. The most common presentation is disseminated disease with fever, weight loss, and night sweats. At least 85% of patients with MAC infection are mycobacteremic, and large numbers of organisms can often be demonstrated on bone marrow biopsy. The chest x-ray is abnormal in ~25% of patients, with the most common pattern being that of a bilateral, lower-lobe infiltrate suggestive of miliary spread. Alveolar or nodular infiltrates and hilar and/or mediastinal adenopathy also can occur. Other clinical findings include endobronchial lesions, abdominal pain, diarrhea, and lymphadenopathy. Anemia and elevated liver alkaline phosphatase are common. The diagnosis is made by the culture of blood or involved tissue. The finding of two consecutive sputum samples positive for MAC is highly suggestive of pulmonary infection. Cultures may take 2 weeks to turn positive. Therapy consists of a macrolide, usually clarithromycin, with ethambutol. Some physicians elect to add a third drug from among rifabutin, ciprofloxacin, or amikacin in patients with extensive disease. Therapy is continued until resolution of clinical signs and symptoms, negative cultures, and CD4+ T cell counts >100/μL for 3–6 months in the setting of cART. Primary prophylaxis for MAC is indicated in patients with HIV infection and CD4+ T cell counts <50/μL (Table 197-11). This may be discontinued in patients in whom cART induces a sustained suppression of viral replication and an increase in CD4+ T cell count to >100/μL for ≥6 months.

Rhodococcus equi is a gram-positive, pleomorphic, acid-fast, nonspore-forming bacillus that can cause pulmonary and/or disseminated infection in patients with advanced HIV infection. Fever and cough are the most common presenting signs. Radiographically one may see cavitary lesions and consolidation. Blood cultures are often positive. Treatment is based on antimicrobial sensitivity testing.

Fungal infections of the lung, in addition to PCP, can be seen in patients with AIDS. Patients with pulmonary cryptococcal disease present with fever, cough, dyspnea, and, in some cases, hemoptysis. A focal or diffuse interstitial infiltrate is seen on chest x-ray in >90% of patients. In addition, one may see lobar disease, cavitary disease, pleural effusions, and hilar or mediastinal adenopathy. More than half of patients are fungemic, and 90% of patients have concomitant CNS infection. *Coccidioides immitis* is a mold that is endemic in the southwest United States. It can cause a reactivation pulmonary syndrome in patients with HIV infection. Most patients with this condition will have CD4+ T cell counts <250/μL. Patients present with fever, weight loss, cough, and extensive, diffuse reticulonodular infiltrates on chest x-ray. One may also see nodules, cavities, pleural effusions, and hilar adenopathy. While serologic testing is of value in the immunocompetent host, serologies are negative in 25% of HIV-infected patients with coccidioidal infection. Invasive aspergillosis is not an AIDS-defining illness and is generally not seen in patients with AIDS in the absence of neutropenia or administration of glucocorticoids. When it does occur, *Aspergillus* infection may have an unusual presentation in the respiratory tract of patients with AIDS, where it gives the appearance of a pseudomembranous tracheobronchitis. Primary pulmonary infection of the lung may be seen with *histoplasmosis*. The most common pulmonary manifestation of histoplasmosis, however, is in the setting of disseminated disease, presumably due to reactivation. In this setting respiratory symptoms are usually minimal, with cough and dyspnea occurring in 10–30% of patients. The chest x-ray is abnormal in ~50% of patients, showing either a diffuse interstitial infiltrate or diffuse small nodules, and the urine will often be positive for *Histoplasma* antigen.

Two forms of *idiopathic interstitial pneumonia* have been identified in patients with HIV infection: lymphoid interstitial pneumonitis (LIP) and nonspecific interstitial pneumonitis (NIP). LIP, a common finding in children, is seen in about 1% of adult patients with untreated HIV infection. This disorder is characterized by a benign infiltrate of the lung and is thought to be part of the polyclonal activation of lymphocytes seen in the context of HIV and EBV infections. Transbronchial biopsy is diagnostic in 50% of the cases, with an open-lung biopsy required for diagnosis in the remainder of cases. This condition is generally self-limited and no specific treatment is necessary. Severe cases have been managed with brief courses of glucocorticoids. Although rarely a clinical problem since the use of cART, evidence of NIP may be seen in up to half of all patients with untreated HIV infection. Histologically, interstitial infiltrates of lymphocytes and plasma cells in a perivascular and peribronchial distribution are present. When symptomatic, patients present with fever and nonproductive cough occasionally accompanied by mild chest discomfort. Chest x-ray is usually normal or may reveal a faint interstitial pattern. Similar to LIP, NIP is a self-limited process for which no therapy is indicated other than appropriate management of the underlying HIV infection. HIV-related pulmonary arterial hypertension (HIV-PAH) is seen in ~0.5% of HIV-infected individuals. Patients may present with an array of symptoms including shortness of breath, fatigue, syncope, chest pain, and signs of right-sided heart failure. Chest x-ray reveals dilated pulmonary vessels and right-sided cardiomegaly with right ventricular hypertrophy seen on electrocardiogram. cART

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does not appear to be of clear benefit, and the prognosis is quite poor with a median survival in the range of 2 years.

Neoplastic diseases of the lung including KS and lymphoma are discussed below in the section on neoplastic diseases.

Diseases of the Cardiovascular System Heart disease is a relatively common postmortem finding in HIV-infected patients (25–75% in autopsy series). The most common form of heart disease is coronary heart disease. In one large series the overall rate of myocardial infarction (MI) was 3.5/1000 patient-years, 28% of these events were fatal, and MI was responsible for 7% of all deaths in the cohort. In patients with HIV infection, cardiovascular disease may be associated with classic risk factors such as smoking, a direct consequence of HIV infection, or a complication of cART. Patients with HIV infection have higher levels of triglycerides, lower levels of high-density lipoprotein cholesterol, and a higher prevalence of smoking than cohorts of individuals without HIV infection. The finding that the rate of cardiovascular disease events was lower in patients on antiretroviral therapy than in those randomized to undergo a treatment interruption identified a clear association between HIV replication and risk of cardiovascular disease. In one study, a baseline CD4+ T cell count of <500/μL was found to be an independent risk factor for cardiovascular disease comparable in magnitude to that attributable to smoking. While the precise pathogenesis of this association remains unclear, it is likely related to the immune activation and increased propensity for coagulation seen as a consequence of HIV replication. Exposure to HIV protease inhibitors and certain reverse transcriptase inhibitors has been associated with increases in total cholesterol and/or risk of MI. Any increases in the risk of death from MI resulting from the use of certain antiretrovirals must be balanced against the marked increases in overall survival brought about by these drugs.

Another form of heart disease associated with HIV infection is a dilated cardiomyopathy associated with congestive heart failure (CHF) referred to as *HIV-associated cardiomyopathy*. This generally occurs as a late complication of HIV infection and, histologically, displays elements of myocarditis. For this reason some have advocated treatment with IV immunoglobulin (IVIg). HIV can be directly demonstrated in cardiac tissue in this setting, and there is debate over whether it plays a direct role in this condition. Patients present with typical findings of CHF including edema and shortness of breath. Patients with HIV infection may also develop cardiomyopathy as side effects of IFN-α or nucleoside analogue therapy. These are reversible once therapy is stopped. KS, cryptococcosis, Chagas' disease, and toxoplasmosis can involve the myocardium, leading to cardiomyopathy. In one series, most patients with HIV infection and a treatable myocarditis were found to have myocarditis associated with toxoplasmosis. Most of these patients also had evidence of CNS toxoplasmosis. Thus, MRI or double-dose contrast CT scan of the brain should be included in the workup of any patient with advanced HIV infection and cardiomyopathy.

A variety of other cardiovascular problems are found in patients with HIV infection. Pericardial effusions may be seen in the setting of advanced HIV infection. Predisposing factors include TB, CHF, mycobacterial infection, cryptococcal infection, pulmonary infection, lymphoma, and KS. While pericarditis is quite rare, in one series 5% of patients with HIV disease had pericardial effusions that were considered to be moderate or severe. Tamponade and death have occurred in association with pericardial KS, presumably owing to acute hemorrhage. Nonbacterial thrombotic endocarditis has been reported and should be considered in patients with unexplained embolic phenomena. IV pentamidine, when given rapidly, can result in hypotension as a consequence of cardiovascular collapse.

Diseases of the Oropharynx and Gastrointestinal System Oropharyngeal and GI diseases are common features of HIV infection. They are most frequently due to secondary infections. In addition, oral and GI lesions may occur with KS and lymphoma.

Oral lesions, including *thrush*, *hairy leukoplakia*, and *aphthous ulcers* (Fig. 197-35), are particularly common in patients with untreated HIV infection. Thrush, due to *Candida* infection, and oral hairy leukoplakia, presumed due to EBV, are usually indicative of fairly advanced immunologic decline; they generally occur in patients with CD4+ T cell counts of <300/μL. In one study, 59% of patients with oral candidiasis went on to develop AIDS in the next year. Thrush appears as a white, cheesy exudate, often on an erythematous mucosa in the posterior oropharynx. While most commonly seen on the soft palate, early lesions are often found along the gingival border. The diagnosis is made by direct examination of a scraping for pseudohyphal elements. Culturing is of no diagnostic value, as patients with HIV infection may have a positive throat culture for *Candida* in the absence of thrush. Oral hairy leukoplakia presents as white, frondlike lesions, generally along the lateral borders of the tongue and sometimes on the adjacent buccal mucosa (Fig. 197-35). Despite its name, oral hairy leukoplakia is not considered a premalignant condition. Lesions are associated with florid replication of EBV. While usually more disconcerting as a sign of HIV-associated immunodeficiency than a clinical problem in need of treatment, severe cases have been reported to respond to topical podophyllin or systemic therapy with anti-herpesvirus agents. Aphthous ulcers of the posterior oropharynx also are seen with regularity in patients with untreated HIV infection (Fig. 197-35). These lesions are of unknown etiology and can be quite painful and interfere with swallowing. Topical anesthetics provide immediate symptomatic relief of short duration. The fact that thalidomide is an effective treatment for this condition suggests that the pathogenesis may involve the action of tissue-destructive cytokines. Palatal, glossal, or gingival ulcers may also result from cryptococcal disease or histoplasmosis.

Esophagitis (Fig. 197-36) may present with odynophagia and retrosternal pain. Upper endoscopy is generally required to make an accurate diagnosis. Esophagitis may be due to *Candida*, CMV, or HSV. While CMV tends to be associated with a single large ulcer, HSV infection is more often associated with multiple small ulcers. The esophagus may also be the site of KS and lymphoma. Like the oral mucosa, the esophageal mucosa may have large, painful ulcers of unclear etiology that may respond to thalidomide. While achlorhydria is a common problem in patients with HIV infection, other gastric problems are generally rare. Among the neoplastic conditions involving the stomach are KS and lymphoma.

Infections of the small and large intestine leading to diarrhea, abdominal pain, and occasionally fever are among the most significant GI problems in HIV-infected patients. They include infections with bacteria, protozoa, and viruses.

Bacteria may be responsible for secondary infections of the GI tract. Infections with enteric pathogens such as *Salmonella*, *Shigella*, and *Campylobacter* are more common in men who have sex with men and are often more severe and more apt to relapse in patients with HIV infection. Patients with untreated HIV have approximately a 20-fold increased risk of infection with *S. typhimurium*. They may present with a variety of nonspecific symptoms including fever, anorexia, fatigue, and malaise of several weeks' duration. Diarrhea is common but may be absent. Diagnosis is made by culture of blood and stool. Long-term therapy with ciprofloxacin is the recommended treatment. HIV-infected patients also have an increased incidence of *S. typhi* infection in areas of the world where typhoid is a problem. *Shigella* spp., particularly *S. flexneri*, can cause severe intestinal disease in HIV-infected individuals. Up to 50% of patients will develop bacteremia. *Campylobacter* infections occur with an increased frequency in patients with HIV infection. While *C. jejuni* is the strain most frequently isolated, infections with many other strains have been reported. Patients usually present with crampy abdominal pain, fever, and bloody diarrhea. Infection may also present as proctitis. Stool examination reveals the presence of fecal leukocytes. Systemic infection can occur, with up to 10% of infected patients exhibiting bacteremia. Most strains are sensitive to erythromycin. Abdominal pain and diarrhea may be seen with MAC infection.

Fungal infections may also be a cause of diarrhea in patients with HIV infection. Histoplasmosis, coccidioidomycosis, and penicilliosis have all been identified as a cause of fever and diarrhea in patients with HIV infection. Peritonitis has been seen with *C. immitis*.

Cryptosporidia, microsporidia, and *Isospora belli* (Chap. 224) are the most common opportunistic protozoa that infect the GI tract and cause diarrhea in HIV-infected patients. Cryptosporidial infection may present in a variety of ways, ranging from a self-limited or intermittent

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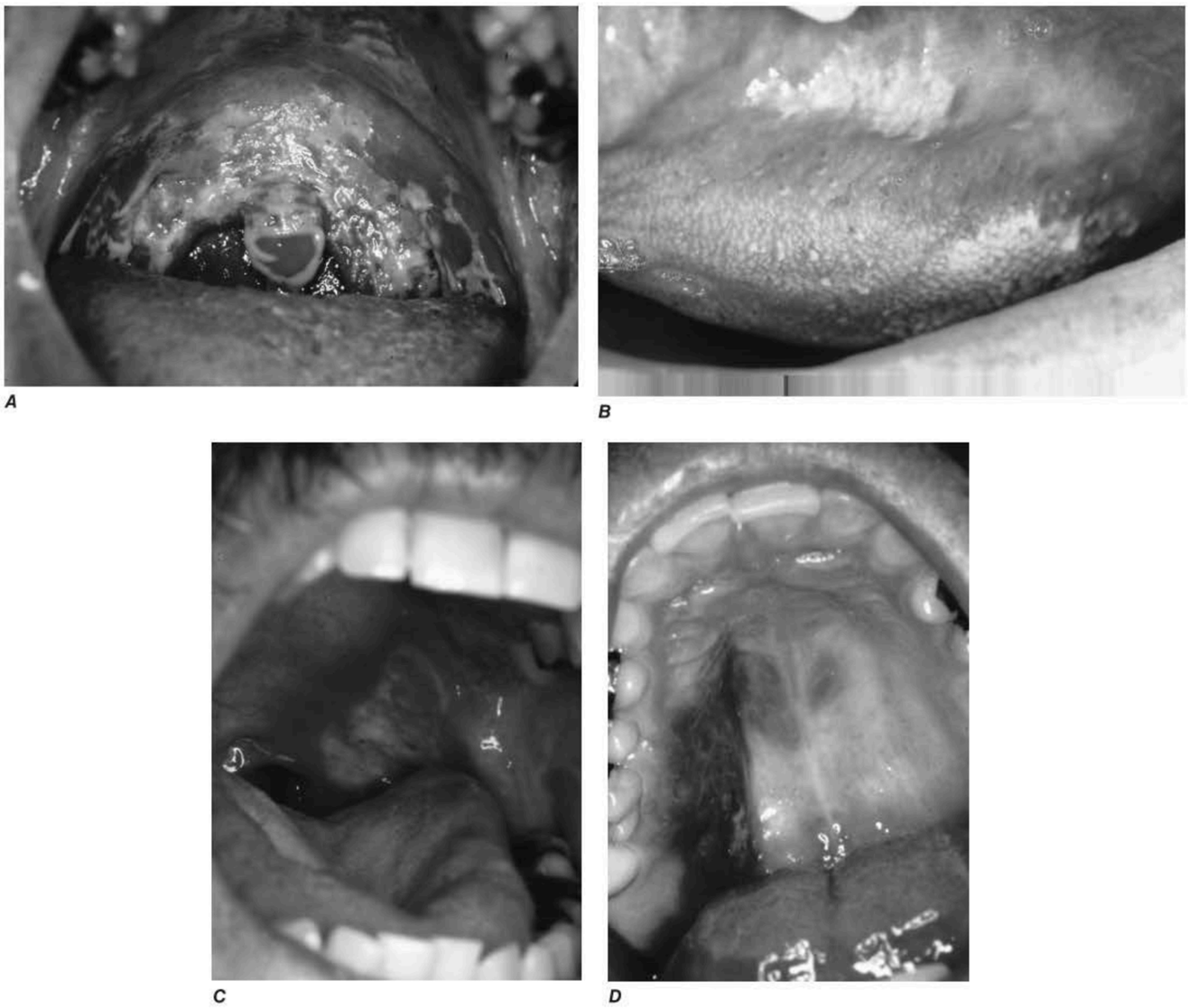

FIGURE 197-35 Various oral lesions in HIV-infected individuals. *A.* Thrush. ***B.*** Hairy leukoplakia. ***C.*** Aphthous ulcer. ***D.*** Kaposi's sarcoma.

diarrheal illness in patients in the early stages of HIV infection to a severe, life-threatening diarrhea in severely immunodeficient individuals. In patients with untreated HIV infection and CD4+ T cell counts of <300/μL, the incidence of cryptosporidiosis is ~1% per year. In 75% of cases the diarrhea is accompanied by crampy abdominal pain, and 25% of patients have nausea and/or vomiting. Cryptosporidia may also cause biliary tract disease in the HIV-infected patient, leading to cholecystitis with or without accompanying cholangitis and pancreatitis secondary to papillary stenosis. The diagnosis of cryptosporidial diarrhea is made by stool examination or biopsy of the small intestine. The diarrhea is noninflammatory, and the characteristic finding is the presence of oocysts that stain with acid-fast dyes. Therapy is predominantly supportive, and marked improvements have been reported in the setting of effective cART. Treatment with up to 2000 mg/d of nitazoxanide (NTZ) is associated with improvement in symptoms or a decrease in shedding of organisms in about half of patients. Its overall role in the management of this condition remains unclear. Patients can minimize their risk of developing cryptosporidiosis by avoiding contact with human and animal feces, by not drinking untreated water from lakes or rivers, and by not eating raw shellfish.

Microsporidia are small, unicellular, obligate intracellular parasites that reside in the cytoplasm of enteric cells **(Chap. 224)**. The main species causing disease in humans is *Enterocytozoon bieneusi*. The clinical manifestations are similar to those described for cryptosporidia and include abdominal pain, malabsorption, diarrhea, and cholangitis. The small size of the organism may make it difficult to detect; however, with the use of chromotrope-based stains, organisms can be identified in stool samples by light microscopy. Definitive diagnosis generally depends on electron-microscopic examination of a stool specimen, intestinal aspirate, or intestinal biopsy specimen. In contrast to cryptosporidia, microsporidia have been noted in a variety of extraintestinal locations, including the eye, brain, sinuses, muscle, and liver, and they have been associated with conjunctivitis and hepatitis. The most effective way to deal with microsporidia in a patient with HIV infection is to restore the immune system by treating the HIV infection with cART. Albendazole, 400 mg bid, has been reported to be of benefit in some patients.

I. belli is a coccidian parasite **(Chap. 224)** most commonly found as a cause of diarrhea in patients from tropical and subtropical regions. Its cysts appear in the stool as large, acid-fast structures that can be differentiated from those of cryptosporidia on the basis of size, shape, and number of sporocysts. The clinical syndromes of *Isospora* infection are identical to those caused by cryptosporidia. The important distinction is that infection with *Isospora* is generally relatively easy to treat with TMP-SMX. While relapses are common, a thrice-weekly regimen of TMP-SMX appears adequate to prevent recurrence.

CMV colitis was once seen as a consequence of advanced immunodeficiency in 5–10% of patients with AIDS. It is much less common with

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FIGURE 197-36 Barium swallow of a patient with *Candida* esophagitis. The flow of barium along the mucosal surface is grossly irregular.

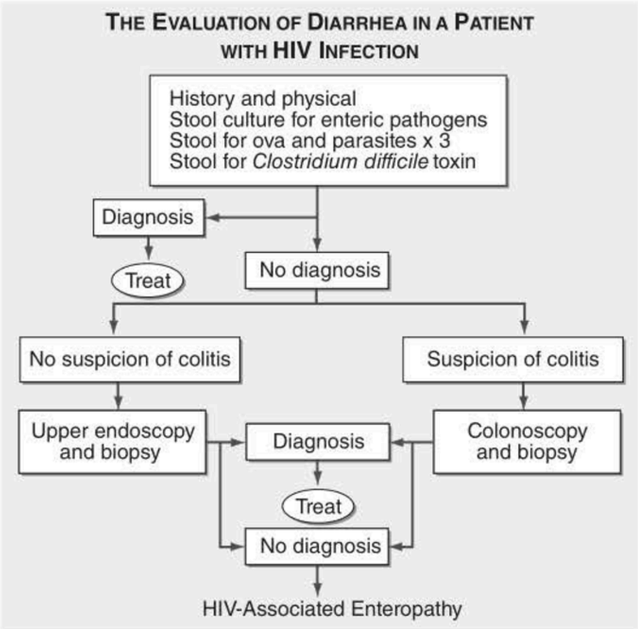

FIGURE 197-37 Algorithm for the evaluation of diarrhea in a patient with HIV infection. HIV-associated enteropathy is a diagnosis of exclusion and can be made only after other, generally treatable, forms of diarrheal illness have been ruled out.

the advent of cART. CMV colitis presents as diarrhea, abdominal pain, weight loss, and anorexia. The diarrhea is usually nonbloody, and the diagnosis is achieved through endoscopy and biopsy. Multiple mucosal ulcerations are seen at endoscopy, and biopsies reveal characteristic intranuclear and cytoplasmic inclusion bodies. Secondary bacteremias may result as a consequence of thinning of the bowel wall. Treatment is with either ganciclovir or foscarnet for 3–6 weeks. Relapses are common, and maintenance therapy is typically necessary in patients whose HIV infection is poorly controlled. Patients with CMV disease of the GI tract should be carefully monitored for evidence of CMV retinitis.

In addition to disease caused by specific secondary infections, patients with HIV infection may also experience a chronic diarrheal syndrome for which no etiologic agent other than HIV can be identified. This entity is referred to as *AIDS enteropathy* or *HIV enteropathy*. It is most likely a direct result of HIV infection in the GI tract. Histologic examination of the small bowel in these patients reveals low-grade mucosal atrophy with a decrease in mitotic figures, suggesting a hyporegenerative state. Patients often have decreased or absent small-bowel lactase and malabsorption with accompanying weight loss.

The initial evaluation of a patient with HIV infection and diarrhea should include a set of stool examinations, including culture, examination for ova and parasites, and examination for *Clostridium difficile* toxin. Approximately 50% of the time this workup will demonstrate infection with pathogenic bacteria, mycobacteria, or protozoa. If the initial stool examinations are negative, additional evaluation, including upper and/or lower endoscopy with biopsy, will yield a diagnosis of microsporidial or mycobacterial infection of the small intestine ~30% of the time. In patients for whom this diagnostic evaluation is nonrevealing, a presumptive diagnosis of HIV enteropathy can be made if the diarrhea has persisted for >1 month. An algorithm for the evaluation of diarrhea in patients with HIV infection is given in Fig. 197-37.

Rectal lesions are common in HIV-infected patients, particularly the perirectal ulcers and erosions due to the reactivation of HSV (Fig. 197-38). These lesions may appear quite atypical, as denuded skin without vesicles. They typically respond well to treatment with valacyclovir, famciclovir, or foscarnet. Other rectal lesions encountered in patients with HIV infection include condylomata acuminata, KS, and intraepithelial neoplasia (see below).

Hepatobiliary Diseases Diseases of the hepatobiliary system are a major problem in patients with HIV infection. It has been estimated that approximately 15% of the deaths of patients with HIV infection are related to liver disease. While this is predominantly a reflection of the problems encountered in the setting of co-infection with hepatitis B or C, it is also a reflection of the hepatic injury, ranging from hepatic steatosis to hypersensitivity reactions to immune reconstitution, that can be seen in the context of cART.

The prevalence of co-infection with HIV and hepatitis viruses varies by geographic region. In the United States, ~90% of HIV-infected individuals have evidence of infection with HBV; 6–14% have chronic HBV infection; 5–50% of patients are co-infected with HCV; and co-infections with hepatitis D, E, and/or G viruses are common. Among IV drug users with HIV infection, rates of HCV infection range from 70% to 95%. HIV infection has a significant impact on the course of hepatitis virus infection. It is associated with approximately a

FIGURE 197-38 Severe, erosive perirectal herpes simplex in a patient with AIDS.

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threefold increase in the development of persistent hepatitis B surface antigenemia. Patients infected with both HBV and HIV have decreased evidence of inflammatory liver disease. The presumption that this is due to the immunosuppressive effects of HIV infection is supported by the observations that this situation can be reversed, and one may see the development of more severe hepatitis following the initiation of effective cART. In studies of the impact of HIV on HBV infection, four- to tenfold increases in liver-related mortality rates have been noted in patients with HIV and active HBV infection compared to rates in patients with either infection alone. There is, however, only a slight increase in overall mortality rate in HIV-infected individuals who are also hepatitis B surface antigen (HBsAg)–positive. IFN-α is less successful as treatment for HBV in patients with HIV co-infection. Lamivudine, emtricitabine, adefovir/tenofovir/entecavir, and telbivudine alone or in combination are useful in the treatment of hepatitis B in patients with HIV infection. It is important to remember that all the above-mentioned drugs also have activity against HIV and should not be used alone in patients with HIV infection, in order to avoid the emergence of quasispecies of HIV resistant to these drugs. For this reason, the treatment of hepatitis B infection in a patient with HIV infection should always be done in the setting of cART. HCV infection is more severe in the patient with HIV infection; it does not appear to affect overall mortality rates in HIV-infected individuals when other variables such as age, baseline CD4+ T cell count, and use of cART are taken into account. In the setting of HIV and HCV co-infection, levels of HCV are approximately tenfold higher than in the HIV-negative patient with HCV infection. There is a 50% higher overall mortality rate with a five-fold increased risk of death due to liver disease in patients chronically infected with both HCV and HIV. Use of directly acting agents for the treatment of HCV leads to cure rates approaching 100%, even in patients with HIV co-infection. Successful treatment of HCV in HIV-infected patients decreases mortality. Hepatitis A virus infection is not seen with an increased frequency in patients with HIV infection. It is recommended that all patients with HIV infection who have not experienced natural infection be immunized with hepatitis A and/or hepatitis B vaccines. Infection with hepatitis G virus, also known as GB virus C, is seen in ~50% of patients with HIV infection. For reasons that are currently unclear, there are data to suggest that patients with HIV infection co-infected with this virus have a decreased rate of progression to AIDS.

A variety of other infections also may involve the liver. Granulomatous hepatitis may be seen as a consequence of mycobacterial or fungal infections, particularly MAC infection. Hepatic masses may be seen in the context of TB, peliosis hepatis, or fungal infection. Among the fungal opportunistic infections, *C. immitis* and *Histoplasma capsulatum* are those most likely to involve the liver. Biliary tract disease in the form of papillary stenosis or sclerosing cholangitis has been reported in the context of cryptosporidiosis, CMV infection, and KS. When no diagnosis can be made, the term *AIDS cholangiopathy* is used. Hemophagocytic lymphohistiocytosis of the liver has been seen in the setting of Hodgkin's disease and may occur prior to diagnosis of the underlying neoplasm.

Many of the drugs used to treat HIV infection are metabolized by the liver and can cause liver injury. Fatal hepatic reactions have been reported with a wide array of antiretrovirals including nucleoside analogues, nonnucleoside analogues, and protease inhibitors. Nucleoside analogues work by inhibiting DNA synthesis. This can result in toxicity to mitochondria, which can lead to disturbances in oxidative metabolism. This may manifest as hepatic steatosis and, in severe cases, lactic acidosis and fulminant liver failure. It is important to be aware of this condition and to watch for it in patients with HIV infection receiving nucleoside analogues. It is reversible if diagnosed early and the offending agent(s) discontinued. Nevirapine has been associated with at times fatal fulminant and cholestatic hepatitis, hepatic necrosis, and hepatic failure. Indinavir may cause mild to moderate elevations in serum bilirubin in 10–15% of patients in a syndrome similar to Gilbert's syndrome. A similar pattern of hepatic injury may be seen with atazanavir. In the patient receiving cART with an unexplained increase in hepatic transaminases, strong consideration should be given to drug toxicity.

Pancreatic injury is most commonly a consequence of drug toxicity, notably that secondary to pentamidine or dideoxynucleosides. While up to half of patients in some series have biochemical evidence of pancreatic injury, <5% of patients show any clinical evidence of pancreatitis that is not linked to a drug toxicity.

Diseases of the Kidney and Genitourinary Tract Diseases of the kidney or genitourinary tract may be a direct consequence of HIV infection, due to an opportunistic infection or neoplasm, or related to drug toxicity. Overall, microalbuminuria is seen in ~20% of untreated HIV-infected patients; significant proteinuria is seen in closer to 2%. The presence of microalbuminuria has been associated with an increase in all-cause mortality rate. *HIV-associated nephropathy* (HIVAN) was first described in IDUs and was initially thought to be IDU nephropathy in patients with HIV infection; it is now recognized as a true direct complication of HIV infection. Although the majority of patients with this condition have CD4+ T cell counts <200/μL, HIV-associated nephropathy can be an early manifestation of HIV infection and is also seen in children. Over 90% of reported cases have been in African-American or Hispanic individuals; the disease is not only more prevalent in these populations but also more severe and is the third leading cause of end-stage renal failure among African Americans age 20–64 in the United States. Proteinuria is the hallmark of this disorder. Edema and hypertension are rare. Ultrasound examination reveals enlarged, hyperechogenic kidneys. A definitive diagnosis is obtained through renal biopsy. Histologically, focal segmental glomerulosclerosis is present in 80%, and mesangial proliferation in 10–15% of cases. Prior to effective antiretroviral therapy, this disease was characterized by relatively rapid progression to end-stage renal disease. Patients with HIV-associated nephropathy should be treated for their HIV infection. Treatment with angiotensin-converting enzyme (ACE) inhibitors and/or prednisone, 60 mg/d, also has been reported to be of benefit in some cases. The incidence of this disease in patients receiving adequate cART has not been well defined; however, the impression is that it has decreased in frequency and severity. It is the leading cause of end-stage renal disease in patients with HIV infection.

Among the drugs commonly associated with renal damage in patients with HIV disease are pentamidine, amphotericin, adefovir, cidofovir, tenofovir, and foscarnet. TMP-SMX may compete for tubular secretion with creatinine and cause an increase in the serum creatinine level. The pharmacokinetic booster cobicistat, a component of several fixed-drug cART formulations, inhibits renal tubular secretion of creatinine leading to increased serum creatinine levels without a true decline in glomerular filtration rate. Sulfadiazine may crystallize in the kidney and result in an easily reversible form of renal shutdown, while indinavir or atazanavir may form renal calculi. Adequate hydration is the mainstay of treatment and prevention for these latter two conditions.

Genitourinary tract infections are seen with a high frequency in patients with HIV infection; they present with skin lesions, dysuria, hematuria, and/or pyuria and are managed in the same fashion as in patients without HIV infection. Infections with HSV are covered below ("Dermatologic Diseases"). Infections with *T. pallidum*, the etiologic agent of *syphilis*, play an important role in the HIV epidemic. In HIV-negative individuals, genital syphilitic ulcers as well as the ulcers of chancroid are major predisposing factors for heterosexual transmission of HIV infection. While most HIV-infected individuals with syphilis have a typical presentation, a variety of formerly rare clinical problems may be encountered in the setting of dual infection. Among them are *lues maligna*, an ulcerating lesion of the skin due to a necrotizing vasculitis; unexplained fever; nephrotic syndrome; and neurosyphilis. The most common presentation of syphilis in the HIV-infected patient is that of *condylomata lata*, a form of secondary syphilis. Neurosyphilis may be asymptomatic or may present as acute meningitis, neuroretinitis, deafness, or stroke. The rate of neurosyphilis may be as high as 1% in patients with HIV infection, and one should consider a lumbar puncture to look for neurosyphilis in all patients with HIV infection and secondary syphilis. As a consequence of the immunologic abnormalities seen in the setting of HIV infection, diagnosis of syphilis

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through standard serologic testing may be challenging. On the one hand, a significant number of patients have false-positive Venereal Disease Research Laboratory (VDRL) tests due to polyclonal B cell activation. On the other hand, the development of a new positive VDRL may be delayed in patients with new infections, and the anti–fluorescent treponemal antibody (anti-FTA) test may be negative due to immunodeficiency. Thus, dark-field examination of appropriate specimens should be performed in any patient in whom syphilis is suspected, even if the patient has a negative VDRL. Similarly, any patient with a positive serum VDRL test, neurologic findings, and an abnormal spinal fluid examination should be considered to have neurosyphilis and treated accordingly, regardless of the CSF VDRL result. In any setting, patients treated for syphilis need to be carefully monitored to ensure adequate therapy. Approximately one-third of patients with HIV infection will experience a Jarisch-Herxheimer reaction upon initiation of therapy for syphilis.

Vulvovaginal candidiasis is a common problem in women with HIV infection. Symptoms include pruritus, discomfort, dyspareunia, and dysuria. Vulvar infection may present as a morbilliform rash that may extend to the thighs. Vaginal infection is usually associated with a white discharge, and plaques may be seen along an erythematous vaginal wall. Diagnosis is made by microscopic examination of the discharge for pseudohyphal elements in a 10% potassium hydroxide solution. Mild disease can be treated with topical therapy. More serious disease can be treated with fluconazole. Other causes of vaginitis include *Trichomonas* and mixed bacteria.

Diseases of the Endocrine System and Metabolic Disorders A variety of endocrine and metabolic disorders are seen in the context of HIV infection. These may be a direct consequence of HIV infection, secondary to opportunistic infections or neoplasms, or related to medication side effects. Between 33% and 75% of patients with HIV infection receiving thymidine analogues or protease inhibitors as a component of cART develop a syndrome often referred to as *lipodystrophy*, consisting of elevations in plasma triglycerides, total cholesterol, and apolipoprotein B, as well as hyperinsulinemia and hyperglycemia. Many of the patients have been noted to have a characteristic set of body habitus changes associated with fat redistribution, consisting of truncal obesity coupled with peripheral wasting (Fig. 197-39). Truncal obesity is apparent as an increase in abdominal girth related to increases in mesenteric fat, a dorsocervical fat pad ("buffalo hump") reminiscent of patients with Cushing's syndrome, and enlargement of the breasts. The peripheral wasting, or lipoatrophy, is particularly noticeable in the face and buttocks and by the prominence of the veins in the legs. These changes may develop at any time ranging from ~6 weeks to several years following the initiation of cART. Approximately 20% of the patients with HIV-associated lipodystrophy meet the criteria for the *metabolic syndrome* as defined by The International Diabetes Federation or The U.S. National Cholesterol Education Program Adult Treatment Panel III. The lipodystrophy syndrome has been reported in association with regimens containing a variety of different drugs, and while initially reported in the setting of protease inhibitor therapy, it appears that similar changes can also be induced by protease-sparing regimens. It has been suggested that the lipoatrophy changes are particularly severe in patients receiving the thymidine analogues stavudine and zidovudine. Current treatment guidelines avoid these drugs and recommend drugs with fewer of these side effects. National Cholesterol Education Program (NCEP) guidelines should be followed in the management of these lipid abnormalities (Chap. 400), and consideration should be given to changing the components of cART with avoidance of thymidine analogues (azidothymidine and stavudine) and offending protease inhibitors. Due to concerns regarding drug interactions, the most commonly utilized lipid-lowering agents in this setting are gemfibrozil and atorvastatin. In addition, lactic acidosis is associated with cART. This is most commonly seen with nucleoside analogue reverse transcriptase inhibitors and can be fatal.

Patients with advanced HIV disease may develop hyponatremia due to the syndrome of inappropriate antidiuretic hormone (vasopressin) secretion (SIADH) as a consequence of increased free-water intake and decreased free-water excretion. SIADH is usually seen in conjunction with pulmonary or CNS disease. Low serum sodium may also be due to adrenal insufficiency; a concomitant high serum potassium should alert one to this possibility. Hyperkalemia may be secondary to adrenal insufficiency; HIV nephropathy; or medications, particularly trimethoprim and pentamidine. Hypokalemia may be seen in the setting of tenofovir or amphotericin therapy. Adrenal gland disease may be due to mycobacterial infections, CMV disease, cryptococcal disease, histoplasmosis, or ketoconazole toxicity. Iatrogenic Cushing's syndrome with suppression of the hypothalamic-pituitary-adrenal axis may be seen with the use of local glucocorticoids (injected or inhaled) in patients receiving ritonavir. This is due to inhibition of the hepatic

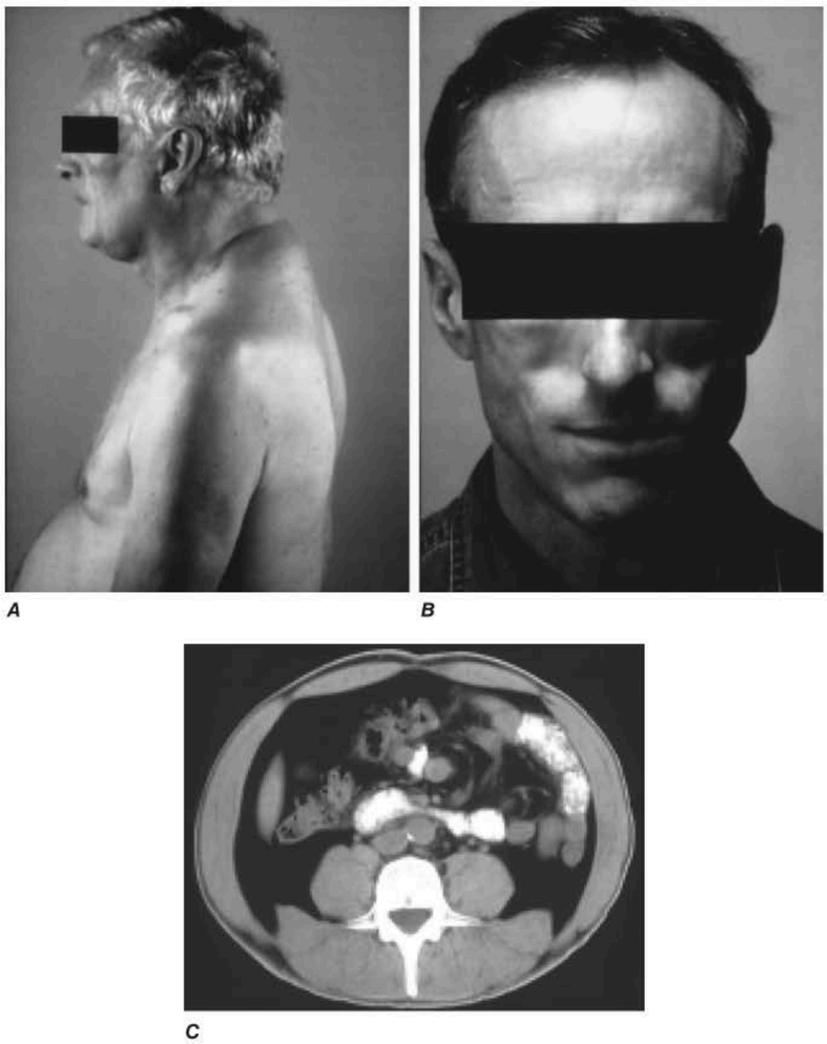

FIGURE 197-39 Characteristics of lipodystrophy. *A.* Truncal obesity and buffalo hump. ***B.*** Facial wasting. ***C.*** Accumulation of intraabdominal fat on CT scan.

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enzyme CYP3A4 by ritonavir leading to prolongation of the glucocorticoid half-life.

Thyroid function may be altered in 10–15% of patients with HIV infection. Both hypo- and hyperthyroidism may be seen. The predominant abnormality is subclinical hypothyroidism. In the setting of cART, up to 10% of patients have been noted to have elevated thyroid-stimulating hormone levels, suggesting that this may be a manifestation of immune reconstitution. Immune-reconstitution Graves' disease may occur as a late (9–48 months) complication of cART. In advanced HIV disease, infection of the thyroid gland may occur with opportunistic pathogens, including *P. jirovecii*, CMV, mycobacteria, *Toxoplasma gondii*, and *Cryptococcus neoformans*. These infections are generally associated with a nontender, diffuse enlargement of the thyroid gland. Thyroid function is usually normal. Diagnosis is made by fine-needle aspirate or open biopsy.

Depending on the severity of disease, HIV infection is associated with *hypogonadism* in 20–50% of men. While this is generally a complication of underlying illness, testicular dysfunction may also be a side effect of ganciclovir therapy. In some surveys, up to two-thirds of patients report decreased libido and one-third complain of erectile dysfunction. Androgen-replacement therapy should be considered in patients with symptomatic hypogonadism. HIV infection does not seem to have a significant effect on the menstrual cycle outside the setting of advanced disease.

Immunologic and Rheumatologic Diseases Immunologic and rheumatologic disorders are common in patients with HIV infection and range from excessive immediate-type hypersensitivity reactions **(Chap. 347)** to an increase in the incidence of reactive arthritis **(Chap. 355)** to conditions characterized by a diffuse infiltrative lymphocytosis. The occurrence of these phenomena is an apparent paradox in the setting of the profound immunodeficiency and immunosuppression that characterizes HIV infection and reflects the complex nature of the immune system and its regulatory mechanisms.

Drug allergies are the most significant allergic reactions occurring in HIV-infected patients and appear to become more common as the disease progresses. They occur in up to 65% of patients who receive therapy with TMP-SMX for PCP. In general, these drug reactions are characterized by erythematous, morbilliform eruptions that are pruritic, tend to coalesce, and are often associated with fever. Nonetheless, ~33% of patients can be maintained on the offending therapy, and thus these reactions are not an immediate indication to stop the drug. Anaphylaxis is extremely rare in patients with HIV infection, and patients who have a cutaneous reaction during a single course of therapy can still be considered candidates for future treatment or prophylaxis with the same agent. The one exception to this is the nucleoside analogue abacavir, where fatal hypersensitivity reactions have been reported with rechallenge. This hypersensitivity is strongly associated with the HLA-B5701 haplotype, and a hypersensitivity reaction to abacavir is an absolute contraindication to future therapy. For other agents, including TMP-SMX, desensitization regimens are moderately successful. While the mechanisms underlying these allergic-type reactions remain unknown, patients with HIV infection have been noted to have elevated IgE levels that increase as the CD4+ T cell count declines. The numerous examples of patients with multiple drug reactions suggest that a common pathway is involved.

HIV infection shares many similarities with a variety of autoimmune diseases, including a substantial polyclonal B cell activation that is associated with a high incidence of antiphospholipid antibodies, such as anticardiolipin antibodies, VDRL antibodies, and lupus-like anticoagulants. In addition, HIV-infected individuals have an increased incidence of antinuclear antibodies. Despite these serologic findings, there is no evidence that HIV-infected individuals have an increase in two of the more common autoimmune diseases, i.e., systemic lupus erythematosus and rheumatoid arthritis. In fact, it has been observed that these diseases may be somewhat ameliorated by the concomitant presence of HIV infection, suggesting that an intact CD4+ T cell limb of the immune response plays an integral role in the pathogenesis of these conditions. Similarly, there are anecdotal reports of patients with common variable immunodeficiency **(Chap. 344)**, characterized by hypogammaglobulinemia, who have had a normalization of Ig levels following the development of HIV infection, suggesting a possible role for overactive CD4+ T cell immunity in certain forms of that syndrome. The one autoimmune disease that may occur with an increased frequency in patients with HIV infection is a variant of primary Sjögren's syndrome **(Chap. 354)**. Patients with HIV infection may develop a syndrome consisting of parotid gland enlargement, dry eyes, and dry mouth that is associated with lymphocytic infiltrates of the salivary gland and lung. One also can see peripheral neuropathy, polymyositis, renal tubular acidosis, and hepatitis. In contrast to Sjögren's syndrome, in which the lymphocytic infiltrates are composed predominantly of CD4+ T cells, in patients with HIV infection the infiltrates are composed predominantly of CD8+ T cells. In addition, while patients with Sjögren's syndrome are mainly women who have autoantibodies to Ro and La and who frequently have HLA-DR3 or B8 MHC haplotypes, HIV-infected individuals with this syndrome are usually African-American men who do not have anti-Ro or anti-La and who most often are HLA-DR5. This syndrome appears to be less common with the increased use of effective cART. The term *diffuse infiltrative lymphocytosis syndrome* (DILS) is used to describe this entity and to distinguish it from Sjögren's syndrome.

Approximately one-third of HIV-infected individuals experience arthralgias; furthermore, 5–10% are diagnosed as having some form of reactive arthritis, such as Reiter's syndrome or psoriatic arthritis as well as undifferentiated spondyloarthropathy **(Chap. 355)**. These syndromes occur with increasing frequency as the competency of the immune system declines. This association may be related to an increase in the number of infections with organisms that may trigger a reactive arthritis with progressive immunodeficiency or to a loss of important regulatory T cells. Reactive arthritides in HIV-infected individuals generally respond well to standard treatment; however, therapy with methotrexate has been associated with an increase in the incidence of opportunistic infections and should be used with caution and only in severe cases.

HIV-infected individuals also experience a variety of joint problems without obvious cause that are referred to generically as *HIV-* or *AIDS-associated arthropathy*. This syndrome is characterized by subacute oligoarticular arthritis developing over a period of 1–6 weeks and lasting 6 weeks to 6 months. It generally involves the large joints, predominantly the knees and ankles, and is nonerosive with only a mild inflammatory response. X-rays are nonrevealing. Nonsteroidal anti-inflammatory drugs are only marginally helpful; however, relief has been noted with the use of intraarticular glucocorticoids. A second form of arthritis also thought to be secondary to HIV infection is called *painful articular syndrome*. This condition, reported as occurring in as many as 10% of AIDS patients, presents as an acute, severe, sharp pain in the affected joint. It affects primarily the knees, elbows, and shoulders; lasts 2–24 h; and may be severe enough to require narcotic analgesics. The cause of this arthropathy is unclear; however, it is thought to result from a direct effect of HIV on the joint. This condition is reminiscent of the fact that other lentiviruses, in particular the caprine arthritis-encephalitis virus, are capable of directly causing arthritis.

A variety of other immunologic or rheumatologic diseases have been reported in HIV-infected individuals, either de novo or in association with opportunistic infections or drugs. Using the criteria of widespread musculoskeletal pain of at least 3 months' duration and the presence of at least 11 of 18 possible tender points by digital palpation, 11% of an HIV-infected cohort containing 55% IDUs were diagnosed as having *fibromyalgia* **(Chap. 366)**. While the incidence of frank arthritis was less in this population than in other studied populations that consisted predominantly of men who have sex with men, these data support the concept that there are musculoskeletal problems that occur as a direct result of HIV infection. In addition there have been reports of leukocytoclastic vasculitis in the setting of zidovudine therapy. CNS angiitis and polymyositis also have been reported in HIV-infected individuals. Septic arthritis is surprisingly rare, especially given the increased incidence of staphylococcal bacteremias seen in this population. When septic arthritis has been reported, it has usually been due

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to *Staphylococcus aureus*, systemic fungal infection with *C. neoformans*, *Sporothrix schenckii*, or *H. capsulatum* or to systemic mycobacterial infection with *M. tuberculosis*, *M. haemophilum*, *M. avium*, or *M. kansasii*.

Patients with HIV infection treated with cART have been found to have an increased incidence of osteonecrosis or avascular necrosis of the hip and shoulders. In a study of asymptomatic patients, 4.4% were found to have evidence of osteonecrosis on MRI. While precise cause-and-effect relationships have been difficult to establish, this complication has been associated with the use of lipid-lowering agents, systemic glucocorticoids, and testosterone; bodybuilding exercise; alcohol consumption; and the presence of anticardiolipin antibodies. Osteoporosis has been reported in 7% of women with HIV infection, with 41% of women demonstrating some degree of osteopenia. Several studies have documented decreases in bone mineral density of 2–6% in the first 2 years following the initiation of cART. This may be particularly apparent with tenofovir-containing regimens.

Immune Reconstitution Inflammatory Syndrome (IRIS) Following the initiation of effective cART, a paradoxical worsening of preexisting, untreated, or partially treated opportunistic infections may be noted. One may also see exacerbations of pre-existing autoimmune conditions or the development of new autoimmune conditions following the initiation of antiretrovirals **(Table 197-12)**. IRIS related to a known pre-existing infection or neoplasm is referred to as *paradoxical IRIS*, while IRIS associated with a previously undiagnosed condition is referred to as *unmasking IRIS*. The term *immune reconstitution disease (IRD)* is sometimes used to distinguish IRIS manifestations related to opportunistic diseases from IRIS manifestations related to autoimmune diseases. IRD is particularly common in patients with underlying untreated mycobacterial or fungal infections. Some form of IRIS is seen in 10–30% of patients, depending on the clinical setting, and is most common in patients starting therapy with CD4+ T cell counts <50 cells/μL who have a precipitous drop in HIV RNA levels following the initiation of cART. Signs and symptoms may appear anywhere from 2 weeks to 2 years after the initiation of cART and can include localized lymphadenitis, prolonged fever, pulmonary infiltrates, hepatitis, increased intracranial pressure, uveitis, sarcoidosis, and Graves' disease. The clinical course can be protracted, and severe cases can be fatal. The underlying mechanism appears to be related to a phenomenon similar to type IV hypersensitivity reactions and reflects the immediate improvements in immune function that occur as levels of HIV RNA drop and the immunosuppressive effects of HIV infection are controlled. In severe cases, the use of immunosuppressive drugs such as glucocorticoids may be required to blunt the inflammatory component of these reactions while specific antimicrobial therapy takes effect.

Diseases of the Hematopoietic System Disorders of the hematopoietic system including lymphadenopathy, anemia, leukopenia, and/or thrombocytopenia are common throughout the course of HIV infection and may be the direct result of HIV, manifestations of secondary infections and neoplasms, or side effects of therapy **(Table 197-13)**. Direct histologic examination and culture of lymph node or bone marrow tissue are often diagnostic. A significant percentage of bone marrow aspirates from patients with HIV infection have been reported to contain lymphoid aggregates, the precise significance of which is unknown. Initiation of cART will lead to reversal of most hematologic complications that are the direct result of HIV infection.

TABLE 197-12 Characteristics of Immune Reconstitution Inflammatory Syndrome (IRIS)

Paradoxical worsening of an existing clinical condition or abrupt appearance of a new clinical finding (unmasking) is seen following the initiation of antiretroviral therapy
Occurs weeks to months following the initiation of antiretroviral therapy
Is most common in patients starting therapy with a CD4+ T cell count <50/μL who experience a precipitous drop in viral load
Is frequently seen in the setting of tuberculosis; particularly when cART is starting soon after initiation of anti-TB therapy
Can be fatal

TABLE 197-13 Causes of Bone Marrow Suppression in Patients with HIV Infection

HIV infection	Medications
Mycobacterial infections	Zidovudine
Fungal infections	Dapsone
B19 parvovirus infection	Trimethoprim/sulfamethoxazole
Lymphoma	Pyrimethamine
	5-Flucytosine
	Ganciclovir
	Interferon α
	Trimetrexate
	Foscarnet

Some patients, otherwise asymptomatic, may develop *persistent generalized lymphadenopathy* as an early clinical manifestation of HIV infection. This condition is defined as the presence of enlarged lymph nodes (>1 cm) in two or more extrainguinal sites for >3 months without an obvious cause. The lymphadenopathy is due to marked follicular hyperplasia in the node in response to HIV infection. The nodes are generally discrete and freely movable. This feature of HIV disease may be seen at any point in the spectrum of immune dysfunction and is not associated with an increased likelihood of developing AIDS. Paradoxically, a loss in lymphadenopathy or a decrease in lymph node size outside the setting of cART may be a prognostic marker of disease progression. In patients with CD4+ T cell counts >200/μL, the differential diagnosis of lymphadenopathy includes KS, TB, Castleman's disease, and lymphoma. In patients with more advanced disease, lymphadenopathy may also be due to atypical mycobacterial infection, toxoplasmosis, systemic fungal infection, or bacillary angiomatosis. While indicated in patients with CD4+ T cell counts <200/μL, lymph node biopsy is not indicated in patients with early-stage disease unless there are signs and symptoms of systemic illness, such as fever and weight loss, or unless the nodes begin to enlarge, become fixed, or coalesce. Monoclonal gammopathy of unknown significance (MGUS) **(Chap. 107)**, defined as the presence of a serum monoclonal IgG, IgA, or IgM in the absence of a clear cause, has been reported in 3% of patients with HIV infection. The overall clinical significance of this finding in patients with HIV infection is unclear, although it has been associated with other viral infections, non-Hodgkin's lymphoma, and plasma cell malignancy.

Anemia is the most common hematologic abnormality in HIV-infected patients and, in the absence of a specific treatable cause, is independently associated with a poor prognosis. While generally mild, anemia can be quite severe and require chronic blood transfusions. Among the specific reversible causes of anemia in the setting of HIV infection are drug toxicity, systemic fungal and mycobacterial infections, nutritional deficiencies, and parvovirus B19 infections. Zidovudine may block erythroid maturation prior to its effects on other marrow elements. A characteristic feature of zidovudine therapy is an elevated mean corpuscular volume (MCV). Another drug used in patients with HIV infection that has a selective effect on the erythroid series is dapsone. This drug can cause a serious hemolytic anemia in patients who are deficient in glucose-6-phosphate dehydrogenase and can create a functional anemia in others through induction of methemoglobinemia. Folate levels are usually normal in HIV-infected individuals; however, vitamin B_{12} levels may be depressed as a consequence of achlorhydria or malabsorption. True autoimmune hemolytic anemia is rare, although ~20% of patients with HIV infection may have a positive direct antiglobulin test as a consequence of polyclonal B cell activation. Infection with parvovirus B19 may also cause anemia. It is important to recognize this possibility given the fact that it responds well to treatment with IVIg. Erythropoietin levels in patients with HIV infection and anemia are generally lower than expected given the degree of anemia. Treatment with erythropoietin may result in an increase in hemoglobin levels. An exception to this is a subset of patients with

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zidovudine-associated anemia in whom erythropoietin levels may be quite high.

During the course of HIV infection, neutropenia may be seen in approximately half of patients. In most instances it is mild; however, it can be severe and can put patients at risk of spontaneous bacterial infections. This is most frequently seen in patients with severely advanced HIV disease and in patients receiving any of a number of potentially myelosuppressive therapies. In the setting of neutropenia, diseases that are not commonly seen in HIV-infected patients, such as aspergillosis or mucormycosis, may occur. Both granulocyte colony-stimulating factor (G-CSF) and GM-CSF increase neutrophil counts in patients with HIV infection regardless of the cause of the neutropenia. Earlier concerns about the potential of these agents to also increase levels of HIV were not confirmed in controlled clinical trials.

Thrombocytopenia may be an early consequence of HIV infection. Approximately 3% of patients with untreated HIV infection and CD4+ T cell counts ≥400/μL have platelet counts <150,000/μL. For untreated patients with CD4+ T cell counts <400/μL, this incidence increases to 10%. In patients receiving antiretrovirals, thrombocytopenia is associated with hepatitis C, cirrhosis, and ongoing high-level HIV replication. Thrombocytopenia is rarely a serious clinical problem in patients with HIV infection and generally responds well to successful cART. Clinically, it resembles the thrombocytopenia seen in patients with idiopathic thrombocytopenic purpura **(Chap. 111)**. Immune complexes containing anti-gp120 antibodies and anti-anti-gp120 antibodies have been noted in the circulation and on the surface of platelets in patients with HIV infection. Patients with HIV infection have also been noted to have a platelet-specific antibody directed toward a 25-kDa component of the surface of the platelet. Other data suggest that the thrombocytopenia in patients with HIV infection may be due to a direct effect of HIV on megakaryocytes. Whatever the cause, it is very clear that the most effective medical approach to this problem has been the use of cART. For patients with platelet counts <20,000/μL, a more aggressive approach combining IVIg or anti-Rh Ig for an immediate response and cART for a more lasting response is appropriate. Rituximab has been used with some success in otherwise refractory cases. Splenectomy is a rarely needed option and is reserved for patients refractory to medical management. Because of the risk of serious infection with encapsulated organisms, all patients with HIV infection about to undergo splenectomy should be immunized with pneumococcal polysaccharide. It should be noted that, in addition to causing an increase in the platelet count, removal of the spleen will result in an increase in the peripheral blood lymphocyte count, making CD4+ T cell counts unreliable markers of immunocompetence. In this setting, the clinician should rely on the CD4+ T cell percentage for making diagnostic decisions with respect to the likelihood of opportunistic infections. A CD4+ T cell percentage of 15 is approximately equivalent to a CD4+ T cell count of 200/μL. In patients with early HIV infection, thrombocytopenia has also been reported as a consequence of classic thrombotic thrombocytopenic purpura **(Chap. 111)**. This clinical syndrome, consisting of fever, thrombocytopenia, hemolytic anemia, and neurologic and renal dysfunction, is a rare complication of early HIV infection. As in other settings, the appropriate management is the use of salicylates and plasma exchange. Other causes of thrombocytopenia include lymphoma, mycobacterial infections, and fungal infections.

The incidence of venous thromboembolic disease such as deep-vein thrombosis or pulmonary embolus is approximately 1% per year in patients with HIV infection. This is approximately 10 times higher than that seen in an age-matched population. Factors associated with an increased risk of clinical thrombosis include age over 45, history of an opportunistic infection, lower CD4 count, and estrogen use. Abnormalities of the coagulation cascade, including decreased protein S activity, increases in factor VIII, anticardiolipin antibodies, PAR-1 expression on T cells, or lupus-like anticoagulant, have been reported in more than 50% of patients with HIV infection. The clinical significance of this increased propensity toward thromboembolic disease is likely reflected in the observation that elevations in D-dimer are strongly associated with all-cause mortality in patients with HIV infection (Table 197-9).

Dermatologic Diseases Dermatologic problems occur in >90% of patients with HIV infection. From the macular, roseola-like rash seen with the acute seroconversion syndrome to extensive end-stage KS, cutaneous manifestations of HIV disease can be seen throughout the course of HIV infection. Among the more common nonneoplastic problems are seborrheic dermatitis, folliculitis, and opportunistic infections. Extrapulmonary pneumocystosis may cause a necrotizing vasculitis. Neoplastic conditions are covered in a separate section below.

Seborrheic dermatitis occurs in 3% of the general population and in up to 50% of patients with HIV infection. Seborrheic dermatitis increases in prevalence and severity as the CD4+ T cell count declines. In HIV-infected patients, seborrheic dermatitis may be aggravated by concomitant infection with *Pityrosporum*, a yeastlike fungus; use of topical antifungal agents has been recommended in cases refractory to standard topical treatment.

Folliculitis is among the most prevalent dermatologic disorders in patients with HIV infection and is seen in ~20% of patients. It is more common in patients with CD4+ T cell counts <200 cells/μL. *Pruritic papular eruption* is one of the most common pruritic rashes in patients with HIV infection. It appears as multiple papules on the face, trunk, and extensor surfaces and may improve with cART. *Eosinophilic pustular folliculitis* is a rare form of folliculitis that is seen with increased frequency in patients with HIV infection. It presents as multiple, urticarial perifollicular papules that may coalesce into plaquelike lesions. Skin biopsy reveals an eosinophilic infiltrate of the hair follicle, which in certain cases has been associated with the presence of a mite. Patients typically have an elevated serum IgE level and may respond to treatment with topical anthelmintics. Pruritus is a common symptom in patients with HIV infection and can lead to *prurigo nodularis*. Patients with HIV infection have also been reported to develop a severe form of *Norwegian scabies* with hyperkeratotic psoriasiform lesions.

Both *psoriasis* and *ichthyosis*, although they are not reported to be increased in frequency, may be particularly severe when they occur in patients with HIV infection. Preexisting psoriasis may become guttate in appearance and more refractory to treatment in the setting of HIV infection.

Reactivation herpes zoster (*shingles*) is seen in 10–20% of patients with HIV infection. This reactivation syndrome of varicella-zoster virus indicates a modest decline in immune function and may be the first indication of clinical immunodeficiency. In one series, patients who developed shingles did so an average of 5 years after HIV infection. In a cohort of patients with HIV infection and localized zoster, the subsequent rate of the development of AIDS was 1% per month. In that study, AIDS was more likely to develop if the outbreak of zoster was associated with severe pain, extensive skin involvement, or involvement of cranial or cervical dermatomes. The clinical manifestations of reactivation zoster in HIV-infected patients, although indicative of immunologic compromise, are not as severe as those seen in other immunodeficient conditions. Thus, while lesions may extend over several dermatomes, involve the spinal cord, and/or be associated with frank cutaneous dissemination, visceral involvement has not been reported. In contrast to patients without a known underlying immunodeficiency state, patients with HIV infection tend to have recurrences of zoster with a relapse rate of ~20%. Valacyclovir, acyclovir, or famciclovir is the treatment of choice. Foscarnet may be of value in patients with acyclovir-resistant virus.

Infection with *herpes simplex virus* in HIV-infected individuals is associated with recurrent orolabial, genital, and perianal lesions as part of recurrent reactivation syndromes **(Chap. 187)**. As HIV disease progresses and the CD4+ T cell count declines, these infections become more frequent and severe. Lesions often appear as beefy red, are exquisitely painful, and have a tendency to occur high in the gluteal cleft (Fig. 197-37). Perirectal HSV may be associated with proctitis and anal fissures. HSV should be high in the differential diagnosis of any HIV-infected patient with a poorly healing, painful perirectal lesion. In addition to recurrent mucosal ulcers, recurrent HSV infection in the form of *herpetic whitlow* can be a problem in patients with HIV infection, presenting with painful vesicles or extensive cutaneous erosion. Valacyclovir, acyclovir, or famciclovir is the treatment of

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choice in these settings. It is noteworthy that even subclinical reactivation of herpes simplex may be associated with increases in plasma HIV RNA levels.

Diffuse skin eruptions due to *Molluscum contagiosum* may be seen in patients with advanced HIV infection. These flesh-colored, umbilicated lesions resemble those of *Penicillium marnefei* or *Cryptococcosis*. They tend to regress with effective cART and can also be treated with local therapy. Similarly, *condyloma acuminatum* lesions may be more severe and more widely distributed in patients with low CD4+ T cell counts. Imiquimod cream may be helpful in some cases. Atypical mycobacterial infections may present as erythematous cutaneous nodules, as may fungal infections, *Bartonella*, *Acanthamoeba*, and KS. Cutaneous infections with *Aspergillus* have been noted at the site of IV catheter placement.

The skin of patients with HIV infection is often a target organ for drug reactions **(Chap. 56)**. Although most skin reactions are mild and not necessarily an indication to discontinue therapy, patients may have particularly severe cutaneous reactions, including erythroderma, *Stevens-Johnson syndrome*, and toxic epidermal necrolysis, as a reaction to drugs—particularly sulfa drugs, nonnucleoside reverse transcriptase inhibitors, abacavir, amprenavir, darunavir, fosamprenavir, and tipranavir. Similarly, patients with HIV infection are often quite photosensitive and burn easily following exposure to sunlight or as a side effect of radiation therapy **(Chap. 57)**.

HIV infection and its treatment may be accompanied by cosmetic changes of the skin that are not of great clinical importance but may be troubling to patients. Yellowing of the nails and straightening of the hair, particularly in African-American patients, have been reported as a consequence of HIV infection. Zidovudine therapy has been associated with elongation of the eyelashes and the development of a bluish discoloration to the nails, again more common in African-American patients. Therapy with clofazimine may cause a yellow-orange discoloration of the skin and urine.

Neurologic Diseases Clinical disease of the nervous system accounts for a significant degree of morbidity in a high percentage of patients with HIV infection **(Table 197-14)**. The neurologic problems that occur in HIV-infected individuals may be either primary to the pathogenic processes of HIV infection or secondary to opportunistic infections or neoplasms. Among the more frequent opportunistic diseases that involve the CNS are toxoplasmosis, cryptococcosis, progressive multifocal leukoencephalopathy, and primary CNS lymphoma. Other less common problems include mycobacterial infections; syphilis; and infection with CMV, herpes zoster, HTLV-1, *Trypanosoma cruzi*, or *Acanthamoeba*. Overall, secondary diseases of the CNS have been reported to occur in approximately one-third of patients with AIDS. These data antedate the widespread use of cART, and this frequency is considerably lower in patients receiving effective antiretroviral drugs.

TABLE 197-14 Neurologic Diseases in Patients with HIV Infection

Opportunistic infections	HIV-1 infection
Toxoplasmosis	Aseptic meningitis
Cryptococcosis	HIV-associated neurocognitive disorders (HAND), including HIV encephalopathy/AIDS dementia complex
Progressive multifocal leukoencephalopathy	Myelopathy
Cytomegalovirus	Vacuolar myelopathy
Syphilis	Pure sensory ataxia
Mycobacterium tuberculosis	Paresthesia/dysesthesia
HTLV-1 infection	Peripheral neuropathy
Amebiasis	Acute inflammatory demyelinating polyneuropathy (Guillain-Barré syndrome)
Neoplasms	Chronic inflammatory demyelinating polyneuropathy (CIDP)
Primary CNS lymphoma	Mononeuritis multiplex
Kaposi's sarcoma	Distal symmetric polyneuropathy
	Myopathy

Primary processes related to HIV infection of the nervous system are reminiscent of those seen with other lentiviruses, such as the maedi-visna virus of sheep.

Neurologic problems directly attributable to HIV occur throughout the course of infection and may be inflammatory, demyelinating, or degenerative in nature. The term *HIV-associated neurocognitive disorders* (HAND) is used to describe a spectrum of disorders that range from asymptomatic neurocognitive impairment (ANI) to minor neurocognitive disorder (MND) to clinically severe dementia. The most severe form, *HIV-associated dementia* (HAD), also referred to as the *AIDS dementia complex*, or *HIV encephalopathy*, is considered an AIDS-defining illness. Most HIV-infected patients have some neurologic problem during the course of their disease. Even in the setting of suppressive cART, approximately 50% of HIV-infected individuals can be shown to have mild to moderate neurocognitive impairment using sensitive neuropsychiatric testing. As noted in the section on pathogenesis, damage to the CNS may be a direct result of viral infection of the CNS macrophages or glial cells or may be secondary to the release of neurotoxins and potentially toxic cytokines such as IL-1β, TNF-α, IL-6, and TGF-β. It has been reported that HIV-infected individuals with the E4 allele for apoE are at increased risk for AIDS encephalopathy and peripheral neuropathy. Virtually all patients with HIV infection have some degree of nervous system involvement with the virus. This is evidenced by the fact that CSF findings are abnormal in ~90% of untreated patients, even during the asymptomatic phase of HIV infection. CSF abnormalities include pleocytosis (50–65% of patients), detection of viral RNA (~75%), elevated CSF protein (35%), and evidence of intrathecal synthesis of anti-HIV antibodies (90%). It is important to point out that evidence of infection of the CNS with HIV does not imply impairment of cognitive function. The neurologic function of an HIV-infected individual should be considered normal unless clinical signs and symptoms suggest otherwise.

Aseptic meningitis may be seen in any but the very late stages of HIV infection. In the setting of acute primary infection, patients may experience a syndrome of headache, photophobia, and meningismus. Rarely, an acute encephalopathy due to encephalitis may occur. Cranial nerve involvement may be seen, predominantly cranial nerve VII but occasionally V and/or VIII. CSF findings include a lymphocytic pleocytosis, elevated protein level, and normal glucose level. This syndrome, which cannot be clinically differentiated from other viral meningitides **(Chap. 134)**, usually resolves spontaneously within 2–4 weeks; however, in some patients, signs and symptoms may become chronic. Aseptic meningitis may occur any time in the course of HIV infection; however, it is rare following the development of AIDS. This suggests that clinical aseptic meningitis in the context of HIV infection is an immune-mediated disease.

Cryptococcus is the leading infectious cause of meningitis in patients with AIDS **(Chap. 210)**. While the vast majority of these are due to *C. neoformans*, up to 12% may be due to *C. gattii*. Cryptococcal meningitis is the initial AIDS-defining illness in ~2% of patients and generally occurs in patients with CD4+ T cell counts <100/μL. Cryptococcal meningitis is particularly common in untreated patients with AIDS in Africa, occurring in ~5% of patients. Most patients present with a picture of subacute meningoencephalitis with fever, nausea, vomiting, altered mental status, headache, and meningeal signs. The incidence of seizures and focal neurologic deficits is low. The CSF profile may be normal or may show only modest elevations in WBC or protein levels and decreases in glucose. The opening pressure in the CSF is usually elevated. In addition to meningitis, patients may develop cryptococcomas and cranial nerve involvement. Approximately one-third of patients also have pulmonary disease. Uncommon manifestations of cryptococcal infection include skin lesions that resemble *molluscum contagiosum*, lymphadenopathy, palatal and glossal ulcers, arthritis, gastroenteritis, myocarditis, and prostatitis. The prostate gland may serve as a reservoir for smoldering cryptococcal infection. The diagnosis of cryptococcal meningitis is made by identification of organisms in spinal fluid with india ink examination or by the detection of cryptococcal antigen. Blood cultures for fungus are often positive. A biopsy may be needed to make a diagnosis of CNS cryptococcoma. Treatment is with

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IV amphotericin B 0.7 mg/kg daily, or liposomal amphotericin 4–6 mg/kg daily, with flucytosine 25 mg/kg qid for at least 2 weeks if possible, continuing with amphotericin alone ideally until the CSF culture turns negative. Decreases in renal function in association with amphotericin can lead to increases in flucytosine levels and subsequent bone marrow suppression. Amphotericin is followed by fluconazole 400 mg/d PO for 8 weeks, and then fluconazole 200 mg/d until the CD4+ T cell count has increased to >200 cells/μL for 6 months in response to cART. Repeated lumbar puncture may be required to manage increased intracranial pressure. Symptoms may recur with initiation of cART as an immune reconstitution syndrome (see above). Other fungi that may cause meningitis in patients with HIV infection are *C. immitis* and *H. capsulatum*. Meningoencephalitis has also been reported due to *Acanthamoeba* or *Naegleria*.

HIV-associated dementia consists of a constellation of signs and symptoms of CNS disease. While this is generally a late complication of HIV infection that progresses slowly over months, it can be seen in patients with CD4+ T cell counts >350 cells/μL. A major feature of this entity is the development of dementia, defined as a decline in cognitive ability from a previous level. It may present as impaired ability to concentrate, increased forgetfulness, difficulty reading, or increased difficulty performing complex tasks. Initially these symptoms may be indistinguishable from findings of situational depression or fatigue. In contrast to "cortical" dementia (such as Alzheimer's disease), aphasia, apraxia, and agnosia are uncommon, leading some investigators to classify HIV encephalopathy as a "subcortical dementia" characterized by defects in short-term memory and executive function (see below). In addition to dementia, patients with HIV encephalopathy may also have motor and behavioral abnormalities. Among the motor problems are unsteady gait, poor balance, tremor, and difficulty with rapid alternating movements. Increased tone and deep tendon reflexes may be found in patients with spinal cord involvement. Late stages may be complicated by bowel and/or bladder incontinence. Behavioral problems include apathy, irritability, and lack of initiative, with progression to a vegetative state in some instances. Some patients develop a state of agitation or mild mania. These changes usually occur without significant changes in level of alertness. This is in contrast to the finding of somnolence in patients with dementia due to toxic/metabolic encephalopathies.

HIV-associated dementia is the initial AIDS-defining illness in ~3% of patients with HIV infection and thus only rarely precedes clinical evidence of immunodeficiency. Clinically significant encephalopathy eventually develops in ~25% of untreated patients with AIDS. As immunologic function declines, the risk and severity of HIV-associated dementia increases. Autopsy series suggest that 80–90% of patients with HIV infection have histologic evidence of CNS involvement. Several classification schemes have been developed for grading HIV encephalopathy; a commonly used clinical staging system is outlined in **Table 197-15**.

TABLE 197-15 Clinical Staging of Hand According to Frascati Criteria

STAGE	NEUROCOGNITIVE STATUS[a]	FUNCTIONAL STATUS[b]
Asymptomatic	1 SD below mean in 2 cognitive domains	No impairments in activities of daily living
Mild neurocognitive disorder	1 SD below mean in 2 cognitive domains	Impairments in activities of daily living
HIV-associated dementia	2 SD below mean in 2 cognitive domains	Notable impairments in activities of daily living

[a]Neurocognitive testing should include assessment of at least 5 domains, including attention-information processing, language, abstraction-executive, complex perceptual motor skills, memory (including learning and recall), simple motor skills, or sensory perceptual skills. Appropriate norms must be available to establish the number of domains in which performance is below 1 SD. [b]Functional status is typically assessed by self-reporting but might be corroborated by a collateral source. No agreed measures exist for HIV-associated neurocognitive disorder criteria. Note that, for diagnosis of HIV-associated neurocognitive disorder, other causes of dementia must be ruled out and potential confounding effects of substance use or psychiatric illness should be considered.

Source: Adapted from A Antinori et al: Neurology 69:1789, 2007.

The precise cause of HIV-associated dementia remains unclear, although the condition is thought to be a result of a combination of direct effects of HIV on the CNS and associated immune activation. HIV has been found in the brains of patients with HIV encephalopathy by Southern blot, in situ hybridization, PCR, and electron microscopy. Multinucleated giant cells, macrophages, and microglial cells appear to be the main cell types harboring virus in the CNS. Histologically, the major changes are seen in the subcortical areas of the brain and include pallor and gliosis, multinucleated giant cell encephalitis, and vacuolar myelopathy. Less commonly, diffuse or focal spongiform changes occur in the white matter. Areas of the brain involved in motor function, language, and judgment are most severely affected.

There are no specific criteria for a diagnosis of HIV-associated dementia, and this syndrome must be differentiated from a number of other diseases that affect the CNS of HIV-infected patients (Table 197-14). The diagnosis of dementia depends on demonstrating a decline in cognitive function. This can be accomplished objectively with the use of a Mini-Mental Status Examination (MMSE) in patients for whom prior scores are available. For this reason, it is advisable for all patients with a diagnosis of HIV infection to have a baseline MMSE. However, changes in MMSE scores may be absent in patients with mild HIV encephalopathy. Imaging studies of the CNS, by either MRI or CT, often demonstrate evidence of cerebral atrophy **(Fig. 197-40)**. MRI may also reveal small areas of increased density on T2-weighted images. Lumbar puncture is an important element of the evaluation of patients with HIV infection and neurologic abnormalities. It is generally most helpful in ruling out or making a diagnosis of opportunistic infections. In HIV encephalopathy, patients may have the nonspecific findings of an increase in CSF cells and protein level. While HIV RNA can often be detected in the spinal fluid and HIV can be cultured from the CSF, this finding is not specific for HIV encephalopathy. There appears to be no correlation between the presence of HIV in the CSF and the presence of HIV encephalopathy. Elevated levels of macrophage chemoattractant protein (MCP-1), β_2-microglobulin, neopterin, and quinolinic acid (a metabolite of tryptophan reported to cause CNS injury) have been noted in the CSF of patients with HIV encephalopathy. These findings suggest that these factors as well as inflammatory cytokines may be involved in the pathogenesis of this syndrome.

Combination antiretroviral therapy is of benefit in patients with HIV-associated dementia. Improvement in neuropsychiatric test scores has been noted for both adult and pediatric patients treated with antiretrovirals. The rapid improvement in cognitive function noted with the initiation of cART suggests that at least some component of this

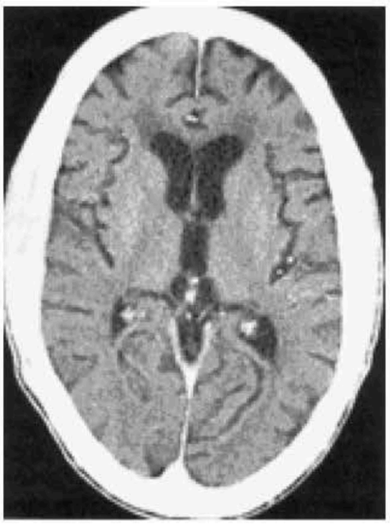

FIGURE 197-40 AIDS dementia complex. Postcontrast CT scan through the lateral ventricles of a 47-year-old man with AIDS, altered mental status, and dementia. The lateral and third ventricles and the cerebral sulci are abnormally prominent. Mild white matter hypodensity is seen adjacent to the frontal horns of the lateral ventricles.

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TABLE 197-16 Causes of Seizures in Patients with HIV Infection

DISEASE	OVERALL CONTRIBUTION TO FIRST SEIZURE, %	FRACTION OF PATIENTS WHO HAVE SEIZURES, %
HIV encephalopathy	24–47	7–50
Cerebral toxoplasmosis	28	15–40
Cryptococcal meningitis	13	8
Primary central nervous system lymphoma	4	15–30
Progressive multifocal leukoencephalopathy	1	20

Source: From DM Holtzman et al: Am J Med 87:173, 1989.

problem is quickly reversible, again supporting at least a partial role of soluble mediators in the pathogenesis. It should also be noted that these patients have an increased sensitivity to the side effects of neuroleptic drugs. The use of these drugs for symptomatic treatment is associated with an increased risk of extrapyramidal side effects; therefore, patients with HIV encephalopathy who receive these agents must be monitored carefully. It is felt by many physicians that the decrease in the prevalence of severe cases of HAND brought about by cART has resulted in an increase in the prevalence of milder forms of this disorder.

Seizures may be a consequence of opportunistic infections, neoplasms, or HIV encephalopathy **(Table 197-16)**. The seizure threshold is often lower than normal in patients with advanced HIV infection due in part to the frequent presence of electrolyte abnormalities. Seizures are seen in 15–40% of patients with cerebral toxoplasmosis, 15–35% of patients with primary CNS lymphoma, 8% of patients with cryptococcal meningitis, and 7–50% of patients with HIV encephalopathy. Seizures may also be seen in patients with CNS tuberculosis, aseptic meningitis, and progressive multifocal leukoencephalopathy. Seizures may be the presenting clinical symptom of HIV disease. In one study of 100 patients with HIV infection presenting with a first seizure, cerebral mass lesions were the most common cause, responsible for 32 of the 100 new-onset seizures. Of these 32 cases, 28 were due to toxoplasmosis and 4 to lymphoma. HIV encephalopathy accounted for an additional 24 new-onset seizures. Cryptococcal meningitis was the third most common diagnosis, responsible for 13 of the 100 seizures. In 23 cases, no cause could be found, and it is possible that these cases represent a subcategory of HIV encephalopathy. Of these 23 cases, 16 (70%) had 2 or more seizures, suggesting that anticonvulsant therapy is indicated in all patients with HIV infection and seizures unless a rapidly correctable cause is found. Due to a variety of drug-drug interactions between antiseizure medications and antiretrovirals, drug levels need to be monitored carefully.

Patients with HIV infection may present with *focal neurologic deficits* from a variety of causes. The most common causes are toxoplasmosis, progressive multifocal leukoencephalopathy, and CNS lymphoma. Other causes include cryptococcal infections (discussed above; also **Chap. 210**), stroke, and reactivation of Chagas' disease.

Toxoplasmosis has been one of the most common causes of secondary CNS infections in patients with AIDS, but its incidence is decreasing in the era of cART. It is most common in patients from the Caribbean and from France, where the seroprevalence of *T. gondii* is around 50%. This figure is closer to 15% in the United States. Toxoplasmosis is generally a late complication of HIV infection and usually occurs in patients with CD4+ T cell counts <200/μL. Cerebral toxoplasmosis is thought to represent a reactivation of latent tissue cysts. It is 10 times more common in patients with antibodies to the organism than in patients who are seronegative. Patients diagnosed with HIV infection should be screened for IgG antibodies to *T. gondii* during the time of their initial workup. Those who are seronegative should be counseled about ways to minimize the risk of primary infection including avoiding the consumption of undercooked meat and careful hand washing after contact with soil or changing the cat litter box. The most common clinical presentation of cerebral toxoplasmosis in patients with HIV infection is fever, headache, and focal neurologic deficits. Patients may present with seizure, hemiparesis, or aphasia as a manifestation of these focal

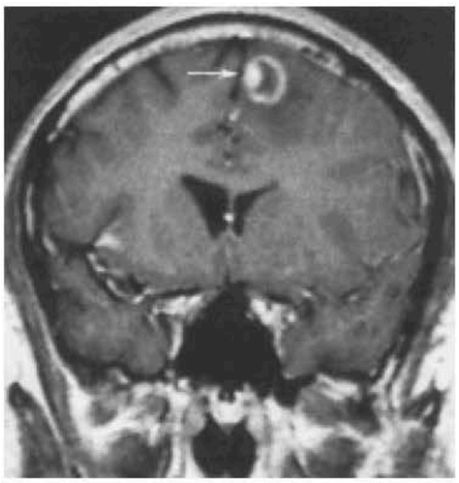

FIGURE 197-41 Central nervous system toxoplasmosis. A coronal postcontrast T1-weighted MRI scan demonstrates a peripheral enhancing lesion in the left frontal lobe, associated with an eccentric nodular area of enhancement (*arrow*); this so-called eccentric target sign is typical of toxoplasmosis.

deficits or with a picture more influenced by the accompanying cerebral edema and characterized by confusion, dementia, and lethargy, which can progress to coma. The diagnosis is usually suspected on the basis of MRI findings of multiple lesions in multiple locations, although in some cases only a single lesion is seen. Pathologically, these lesions generally exhibit inflammation and central necrosis and, as a result, demonstrate ring enhancement on contrast MRI **(Fig. 197-41)** or, if MRI is unavailable or contraindicated, on double-dose contrast CT. There is usually evidence of surrounding edema. In addition to toxoplasmosis, the differential diagnosis of single or multiple enhancing mass lesions in the HIV-infected patient includes primary CNS lymphoma and, less commonly, TB or fungal or bacterial abscesses. The definitive diagnostic procedure is brain biopsy. However, given the morbidity rate that can accompany this procedure, it is usually reserved for the patient who has failed 2–4 weeks of empiric therapy for toxoplasmosis. If the patient is seronegative for *T. gondii*, the likelihood that a mass lesion is due to toxoplasmosis is <10%. In that setting, one may choose to be more aggressive and perform a brain biopsy sooner. Standard treatment is sulfadiazine and pyrimethamine with leucovorin as needed for a minimum of 4–6 weeks. Alternative therapeutic regimens include clindamycin in combination with pyrimethamine; atovaquone plus pyrimethamine; and azithromycin plus pyrimethamine plus rifabutin. Relapses are common, and it is recommended that patients with a history of prior toxoplasmic encephalitis receive maintenance therapy with sulfadiazine, pyrimethamine, and leucovorin as long as their CD4+ T cell counts remain <200 cells/μL. Patients with CD4+ T cell counts <100/μL and IgG antibody to *Toxoplasma* should receive primary prophylaxis for toxoplasmosis. Fortunately, the same daily regimen of a single double-strength tablet of TMP-SMX used for *P. jirovecii* prophylaxis provides adequate primary protection against toxoplasmosis. Secondary prophylaxis/maintenance therapy for toxoplasmosis may be discontinued in the setting of effective cART and increases in CD4+ T cell counts to >200/μL for 6 months.

JC virus, a human polyomavirus that is the etiologic agent of *progressive multifocal leukoencephalopathy* (PML), is an important opportunistic pathogen in patients with AIDS **(Chap. 133)**. While ~80% of the general adult population has antibodies to JC virus, indicative of prior infection, <10% of healthy adults show any evidence of ongoing viral replication. PML is the only known clinical manifestation of JC virus infection. It is a late manifestation of AIDS and is seen in ~1–4% of patients with AIDS. The lesions of PML begin as small foci of demyelination in subcortical white matter that eventually coalesce. The cerebral hemispheres, cerebellum, and brainstem may all be involved. Patients typically have a protracted course with multifocal neurologic deficits, with or without changes in mental status. Approximately 20% of patients experience seizures. Ataxia, hemiparesis, visual field

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defects, aphasia, and sensory defects may occur. Headache, fever, nausea, and vomiting are rarely seen. Their presence should suggest another diagnosis. MRI typically reveals multiple, nonenhancing white matter lesions that may coalesce and have a predilection for the occipital and parietal lobes. The lesions show signal hyperintensity on T2-weighted images and diminished signal on T1-weighted images. The measurement of JC virus DNA levels in CSF has a diagnostic sensitivity of 76% and a specificity of close to 100%. Prior to the availability of cART, the majority of patients with PML died within 3–6 months of the onset of symptoms. Paradoxical worsening of PML has been seen with initiation of cART as an immune reconstitution syndrome. There is no specific treatment for PML; however, a median survival of 2 years and survival of >15 years have been reported in patients with PML treated with cART for their HIV disease. Despite having a significant impact on survival, only ~50% of patients with HIV infection and PML show neurologic improvement with cART. Studies with other antiviral agents such as cidofovir have failed to show clear benefit. Factors influencing a favorable prognosis for PML in the setting of HIV infection include a CD4+ T cell count >100/μL at baseline and the ability to maintain an HIV viral load of <500 copies/mL. Baseline HIV-1 viral load does not have independent predictive value of survival. PML is one of the few opportunistic infections that continues to occur with some frequency despite the widespread use of cART.

Reactivation American trypanosomiasis may present as acute meningoencephalitis with focal neurologic signs, fever, headache, vomiting, and seizures. Accompanying cardiac disease in the form of arrhythmias or heart failure should increase the index of suspicion. The presence of antibodies to *T. cruzi* supports the diagnosis. In South America, reactivation of *Chagas' disease* is considered to be an AIDS-defining condition and may be the initial AIDS-defining condition. The majority of cases occur in patients with CD4+ T cell counts <200 cells/μL. Lesions appear radiographically as single or multiple hypodense areas, typically with ring enhancement and edema. They are found predominantly in the subcortical areas, a feature that differentiates them from the deeper lesions of toxoplasmosis. *T. cruzi* amastigotes, or trypanosomes, can be identified from biopsy specimens or CSF. Other CSF findings include elevated protein and a mild (<100 cells/μL) lymphocytic pleocytosis. Organisms can also be identified by direct examination of the blood. Treatment consists of benzimidazole (2.5 mg/kg bid) or nifurtimox (2 mg/kg qid) for at least 60 days, followed by maintenance therapy for the duration of immunodeficiency with either drug at a dose of 5 mg/kg three times a week. As is the case with cerebral toxoplasmosis, successful therapy with antiretrovirals may allow discontinuation of therapy for Chagas' disease.

Stroke may occur in patients with HIV infection. In contrast to the other causes of focal neurologic deficits in patients with HIV infection, the symptoms of a stroke are sudden in onset. Patients with HIV infection have an increased prevalence of many classic risk factors associated with stroke, including smoking and diabetes. It has been reported that HIV infection itself can lead to an increase in carotid artery stiffness. The relative increase in risk for stroke as a consequence of HIV infection is more pronounced in women and in individuals between the ages of 18 and 29. Among the secondary infectious diseases in patients with HIV infection that may be associated with stroke are vasculitis due to cerebral varicella zoster or neurosyphilis and septic embolism in association with fungal infection. Other elements of the differential diagnosis of stroke in the patient with HIV infection include atherosclerotic cerebral vascular disease, thrombotic thrombocytopenic purpura, and cocaine or amphetamine use.

Primary CNS lymphoma is discussed below in the section on neoplastic diseases.

Spinal cord disease, or myelopathy, is present in ~20% of patients with AIDS, often as part of HIV-associated neurocognitive disorder. In fact, 90% of the patients with HIV-associated myelopathy have some evidence of dementia, suggesting that similar pathologic processes may be responsible for both conditions. Three main types of spinal cord disease are seen in patients with AIDS. The first of these is a vacuolar myelopathy, as mentioned above. This condition is pathologically similar to subacute combined degeneration of the cord, such as that occurring with pernicious anemia. Although vitamin B_{12} deficiency can be seen in patients with AIDS as a primary complication of HIV infection, it does not appear to be responsible for the majority of the cases of myelopathy seen in patients with HIV infection. Vacuolar myelopathy is characterized by a subacute onset and often presents with gait disturbances, predominantly ataxia and spasticity; it may progress to include bladder and bowel dysfunction. Physical findings include evidence of increased deep tendon reflexes and extensor plantar responses. The second form of spinal cord disease involves the dorsal columns and presents as a pure sensory ataxia. The third form is also sensory in nature and presents with paresthesias and dysesthesias of the lower extremities. In contrast to the cognitive problems seen in patients with HIV encephalopathy, these spinal cord syndromes do not respond well to antiretroviral drugs, and therapy is mainly supportive.

One important disease of the spinal cord that also involves the peripheral nerves is a *myelopathy* and *polyradiculopathy* seen in association with CMV infection. This entity is generally seen late in the course of HIV infection and is fulminant in onset, with lower extremity and sacral paresthesias, difficulty in walking, areflexia, ascending sensory loss, and urinary retention. The clinical course is rapidly progressive over a period of weeks. CSF examination reveals a predominantly neutrophilic pleocytosis, and CMV DNA can be detected by CSF PCR. Therapy with ganciclovir or foscarnet can lead to rapid improvement, and prompt initiation of foscarnet or ganciclovir therapy is important in minimizing the degree of permanent neurologic damage. Combination therapy with both drugs should be considered in patients who have been previously treated for CMV disease. Other diseases involving the spinal cord in patients with HIV infection include HTLV-1-associated myelopathy (HAM) **(Chap. 196)**, neurosyphilis **(Chap. 177)**, infection with herpes simplex **(Chap. 187)** or varicella-zoster **(Chap. 188)**, TB **(Chap. 173)**, and lymphoma **(Chap. 104)**.

Peripheral neuropathies are common in patients with HIV infection. They occur at all stages of illness and take a variety of forms. Early in the course of HIV infection, an acute inflammatory demyelinating polyneuropathy resembling Guillain-Barré syndrome may occur **(Chap. 439)**. In other patients, a progressive or relapsing-remitting inflammatory neuropathy resembling chronic inflammatory demyelinating polyneuropathy (CIDP) has been noted. Patients commonly present with progressive weakness, areflexia, and minimal sensory changes. CSF examination often reveals a mononuclear pleocytosis, and peripheral nerve biopsy demonstrates a perivascular infiltrate suggesting an autoimmune etiology. Plasma exchange or IVIg has been tried with variable success. Because of the immunosuppressive effects of glucocorticoids, they should be reserved for severe cases of CIDP refractory to other measures. Another autoimmune peripheral neuropathy seen in patients with AIDS is mononeuritis multiplex **(Chaps. 439 and 356)** due to a necrotizing arteritis of peripheral nerves. The most common peripheral neuropathy in patients with HIV infection is a *distal sensory polyneuropathy* (DSPN) also referred to as painful sensory neuropathy (HIV-SN), predominantly sensory neuropathy, or distal symmetric peripheral neuropathy. This condition may be a direct consequence of HIV infection or a side effect of dideoxynucleoside therapy. It is more common in taller individuals, older individuals, and those with lower CD4 counts. Two-thirds of patients with AIDS may be shown by electrophysiologic studies to have some evidence of peripheral nerve disease. Presenting symptoms are usually painful burning sensations in the feet and lower extremities. Findings on examination include a stocking-type sensory loss to pinprick, temperature, and touch sensation and a loss of ankle reflexes. Motor changes are mild and are usually limited to weakness of the intrinsic foot muscles. Response of this condition to antiretrovirals has been variable, perhaps because antiretrovirals are responsible for the problem in some instances. When due to dideoxynucleoside therapy, patients with lower extremity peripheral neuropathy may complain of a sensation that they are walking on ice. Other entities in the differential diagnosis of peripheral neuropathy include diabetes mellitus, vitamin B_{12} deficiency, and side effects from metronidazole or dapsone. For distal symmetric polyneuropathy that fails to resolve following the discontinuation of dideoxynucleosides, therapy is symptomatic; gabapentin, carbamazepine, tricyclics, or

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analgesics may be effective for dysesthesias. Treatment-naïve patients may respond to cART.

Myopathy may complicate the course of HIV infection; causes include HIV infection itself, zidovudine, and the generalized wasting syndrome (discussed below). HIV-associated myopathy may range in severity from an asymptomatic elevation in creatine kinase levels to a subacute syndrome characterized by proximal muscle weakness and myalgias. Quite pronounced elevations in creatine kinase may occur in asymptomatic patients, particularly after exercise. The clinical significance of this as an isolated laboratory finding is unclear. A variety of both inflammatory and noninflammatory pathologic processes have been noted in patients with more severe myopathy, including myofiber necrosis with inflammatory cells, nemaline rod bodies, cytoplasmic bodies, and mitochondrial abnormalities. Profound muscle wasting, often with muscle pain, may be seen after prolonged zidovudine therapy. This toxic side effect of the drug is dose-dependent and is related to its ability to interfere with the function of mitochondrial polymerases. It is reversible following discontinuation of the drug. Red ragged fibers are a histologic hallmark of zidovudine-induced myopathy.

Ophthalmologic Diseases Ophthalmologic problems occur in ~50% of patients with advanced HIV infection. The most common abnormal findings on funduscopic examination are cotton-wool spots. These are hard white spots that appear on the surface of the retina and often have an irregular edge. They represent areas of retinal ischemia secondary to microvascular disease. At times they are associated with small areas of hemorrhage and thus can be difficult to distinguish from CMV retinitis. In contrast to CMV retinitis, however, these lesions are not associated with visual loss and tend to remain stable or improve over time.

One of the most devastating consequences of HIV infection is CMV retinitis. Patients at high risk of CMV retinitis (CD4+ T cell count <100/μL) should undergo an ophthalmologic examination every 3–6 months. The majority of cases of CMV retinitis occur in patients with a CD4+ T cell count <50/μL. Prior to the availability of cART, this CMV reactivation syndrome was seen in 25–30% of patients with AIDS. In the cART era this has dropped to close to 2%. CMV retinitis usually presents as a painless, progressive loss of vision. Patients may also complain of blurred vision, "floaters," and scintillations. The disease is usually bilateral, although typically it affects one eye more than the other. The diagnosis is made on clinical grounds by an experienced ophthalmologist. The characteristic retinal appearance is that of perivascular hemorrhage and exudate. In situations where the diagnosis is in doubt due to an atypical presentation or an unexpected lack of response to therapy, vitreous or aqueous humor sampling with molecular diagnostic techniques may be of value. CMV infection of the retina results in a necrotic inflammatory process, and the visual loss that develops is irreversible. CMV retinitis may be complicated by rhegmatogenous retinal detachment as a consequence of retinal atrophy in areas of prior inflammation. Therapy for CMV retinitis consists of oral valganciclovir, IV ganciclovir, or IV foscarnet, with cidofovir as an alternative. Combination therapy with ganciclovir and foscarnet has been shown to be slightly more effective than either ganciclovir or foscarnet alone in the patient with relapsed CMV retinitis. A 3-week induction course is followed by maintenance therapy with oral valganciclovir. If CMV disease is limited to the eye, intravitreal injections of ganciclovir or foscarnet may be considered. Intravitreal injections of cidofovir are generally avoided due to the increased risk of uveitis and hypotony. Maintenance therapy is continued until the CD4+ T cell count remains >100 μL for >6 months. The majority of patients with HIV infection and CMV disease develop some degree of uveitis with the initiation of cART. The etiology of this is unknown; however, it has been suggested that this may be due to the generation of an enhanced immune response to CMV as an IRIS (see above). In some instances this has required the use of topical glucocorticoids.

Both HSV and varicella zoster virus can cause a rapidly progressing, bilateral, necrotizing retinitis referred to as the *acute retinal necrosis syndrome*, or *progressive outer retinal necrosis* (PORN). This syndrome, in contrast to CMV retinitis, is associated with pain, keratitis, and iritis. It is often associated with orolabial HSV or trigeminal zoster. Ophthalmologic examination reveals widespread pale gray peripheral lesions. This condition is often complicated by retinal detachment. It is important to recognize and treat this condition with IV acyclovir as quickly as possible to minimize the loss of vision.

Several other secondary infections may cause ocular problems in HIV-infected patients. *P. jirovecii* can cause a lesion of the choroid that may be detected as an incidental finding on ophthalmologic examination. These lesions are typically bilateral, are from half to twice the disc diameter in size, and appear as slightly elevated yellow-white plaques. They are usually asymptomatic and may be confused with cotton-wool spots. Chorioretinitis due to toxoplasmosis can be seen alone or, more commonly, in association with CNS toxoplasmosis. KS may involve the eyelid or conjunctiva, while lymphoma may involve the retina. Syphilis may lead to a uveitis that is highly associated with the presence of neurosyphilis.

Additional Disseminated Infections and Wasting Syndrome Infections with species of the small, gram-negative, *Rickettsia*-like organism *Bartonella* (Chap. 167) are seen with increased frequency in patients with HIV infection. While it is not considered an AIDS-defining illness by the CDC, many experts view infection with *Bartonella* as indicative of a severe defect in cell-mediated immunity. It is usually seen in patients with CD4+ T cell counts <100/μL and is a significant cause of unexplained fever in patients with advanced HIV infection. Among the clinical manifestations of *Bartonella* infection are bacillary angiomatosis, cat-scratch disease, and trench fever. *Bacillary angiomatosis* is usually due to infection with *B. henselae* and is linked to exposure to flea-infested cats. It is characterized by a vascular proliferation that leads to a variety of skin lesions that have been confused with the skin lesions of KS. In contrast to the lesions of KS, the lesions of bacillary angiomatosis generally blanch, are painful, and typically occur in the setting of systemic symptoms. Infection can extend to the lymph nodes, liver (peliosis hepatis), spleen, bone, heart, CNS, respiratory tract, and GI tract. *Cat-scratch disease* also is due to *B. henselae* and generally begins with a papule at the site of inoculation. This is followed several weeks later by the development of regional adenopathy and malaise. Infection with *B. quintana* is transmitted by lice and has been associated with case reports of trench fever, endocarditis, adenopathy, and bacillary angiomatosis. The organism is quite difficult to culture, and diagnosis often relies on identifying the organism in biopsy specimens using the Warthin-Starry or similar stains. Treatment is with either doxycycline or erythromycin for at least 3 months.

Histoplasmosis is an opportunistic infection that is seen most frequently in patients in the Mississippi and Ohio River valleys, Puerto Rico, the Dominican Republic, and South America. These are all areas in which infection with *H. capsulatum* is endemic (Chap. 207). Because of this limited geographic distribution, the percentage of AIDS cases in the United States with histoplasmosis is only ~0.5. Histoplasmosis is generally a late manifestation of HIV infection; however, it may be the initial AIDS-defining condition. In one study, the median CD4+ T cell count for patients with histoplasmosis and AIDS was 33/μL. While disease due to *H. capsulatum* may present as a primary infection of the lung, disseminated disease, presumably due to reactivation, is the most common presentation in HIV-infected patients. Patients usually present with a 4- to 8-week history of fever and weight loss. Hepatosplenomegaly and lymphadenopathy are each seen in about 25% of patients. CNS disease, either meningitis or a mass lesion, is seen in 15% of patients. Bone marrow involvement is common, with thrombocytopenia, neutropenia, and anemia occurring in 33% of patients. Approximately 7% of patients have mucocutaneous lesions consisting of a maculopapular rash and skin or oral ulcers. Respiratory symptoms are usually mild, with chest x-ray showing a diffuse infiltrate or diffuse small nodules in ~50% of cases. The gastrointestinal tract may be involved. Diagnosis is made by silver staining of tissue, by culturing the organisms from blood, bone marrow, or tissue, or by detecting antigen in blood or urine. Treatment is typically with liposomal amphotericin B followed by maintenance therapy with oral itraconazole until the serum histoplasma antigen is <2 units, the patient has been on antiretrovirals for at least 6 months, and the CD4 count is >150 cells/μL. In the setting of mild infection, it may be appropriate to initiate therapy with itraconazole alone.

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Following the spread of HIV infection to southeast Asia, disseminated infection with the fungus *Penicillium marneffei* was recognized as a complication of HIV infection and is considered an AIDS-defining condition in those parts of the world where it occurs. *P. marneffei* is the third most common AIDS-defining illness in Thailand, following TB and cryptococcosis. It is more frequently diagnosed in the rainy than the dry season. Clinical features include fever, generalized lymphadenopathy, hepatosplenomegaly, anemia, thrombocytopenia, and papular skin lesions with central umbilication resembling the lesions of *Molluscum contagiosum*. Treatment is with amphotericin B followed by itraconazole until the CD4+ T cell count is >100 cells/μL for at least 6 months.

Visceral leishmaniasis **(Chap. 221)** is recognized with increasing frequency in patients with HIV infection who live in or travel to areas endemic for this protozoal infection transmitted by sandflies. The clinical presentation is one of hepatosplenomegaly, fever, and hematologic abnormalities. Lymphadenopathy and other constitutional symptoms may be present. A chronic, relapsing course is seen in two-thirds of co-infected patients. Organisms can be isolated from cultures of bone marrow aspirates. Histologic stains may be negative, and antibody titers are of little help. Patients with HIV infection usually respond well initially to standard therapy with amphotericin B or pentavalent antimony compounds. Eradication of the organism is difficult, however, and relapses are common.

Patients with HIV infection are at a slightly increased risk of clinical malaria. This is particularly true for patients from nonendemic areas and thus at risk for primary infection and in patients with lower CD4+ T cell counts. HIV-positive individuals with CD4+ T cell counts <300 cells/μL have a poorer response to malaria treatment than others. Co-infection with malaria is associated with a modest increase in HIV viral load. The risk of malaria may be decreased with TMP-SMX prophylaxis.

Generalized wasting is an AIDS-defining condition; it is defined as involuntary weight loss of >10% associated with intermittent or constant fever and chronic diarrhea or fatigue lasting >30 days in the absence of a defined cause other than HIV infection. Prior to the widespread use of cART it was the initial AIDS-defining condition in ~10% of patients with AIDS in the United States. Generalized wasting is rarely seen today with the earlier initiation of antiretrovirals. A constant feature of this syndrome is severe muscle wasting with scattered myofiber degeneration and occasional evidence of myositis. Glucocorticoids may be of some benefit; however, this approach must be carefully weighed against the risk of compounding the immunodeficiency of HIV infection. Androgenic steroids, growth hormone, and total parenteral nutrition have been used as therapeutic interventions with variable success.

Neoplastic Diseases The neoplastic diseases considered to be AIDS-defining conditions are Kaposi's sarcoma, non-Hodgkin's lymphoma, and invasive cervical carcinoma. In addition, there is also an increase in the incidence of a variety of non-AIDS-defining malignancies including Hodgkin's disease; multiple myeloma; leukemia; melanoma; and cervical, brain, testicular, oral, lung, gastric, liver, renal, and anal cancers. Since the introduction of potent cART, there has been a marked reduction in the incidence of KS (Fig. 197-34). The non-AIDS-defining malignancies now account for more morbidity and mortality in patients with HIV infection than the AIDS-defining malignancies and are responsible for approximately 25% of the deaths in patients with HIV infection. Rates of non-Hodgkin's lymphoma have declined; however, this decline has not been as dramatic as the decline in rates of KS. In contrast, cART has had little effect on human papillomavirus (HPV)-associated malignancies. As patients with HIV infection live longer, a wider array of cancers is seen in this population. While some may only reflect known risk factors (e.g., smoking, alcohol consumption, co-infection with other viruses such as hepatitis B) that are increased in patients with HIV infection, some may be a direct consequence of HIV and are clearly increased in patients with lower CD4+ T cell counts.

Kaposi's sarcoma is a multicentric neoplasm consisting of multiple vascular nodules appearing in the skin, mucous membranes, and viscera. The clinical course of KS ranges from indolent, with only minor skin or lymph node involvement, to fulminant, with extensive cutaneous and visceral involvement. In the initial period of the AIDS epidemic, KS was a prominent clinical feature of the first cases of AIDS, occurring in 79% of the patients diagnosed in 1981. By 1989 it was seen in only 25% of cases, by 1992 the number had decreased to 9%, and by 1997 the number was <1%. HHV-8 (KSHV) has been strongly implicated as a viral cofactor in the pathogenesis of KS.

Clinically, KS has varied presentations and may be seen at any stage of HIV infection, even in the presence of a normal CD4+ T cell count. The initial lesion may be a small, raised reddish-purple nodule on the skin **(Fig. 197-42)**, a discoloration on the oral mucosa (Fig. 197-34*D*), or a swollen lymph node. Lesions often appear in sun-exposed areas, particularly the tip of the nose, and have a propensity to occur in areas of trauma (Koebner phenomenon). Because of the vascular nature of the tumors and the presence of extravasated red blood cells in the lesions, their colors range from reddish to purple to brown and often take the appearance of a bruise, with yellowish discoloration and tattooing. Lesions range in size from a few millimeters to several centimeters in diameter and may be either discrete or confluent. KS lesions most commonly appear as raised macules; however, they can also be papular, particularly in patients with higher CD4+ T cell counts. Confluent lesions may give rise to surrounding lymphedema and may be disfiguring when they involve the face and disabling when they involve the lower extremities or the surfaces of joints. Apart from skin, the lymph nodes, GI tract, and lung are the organ systems most commonly affected by KS. Lesions have been reported in virtually every organ, including the heart and the CNS. In contrast to most malignancies, in which lymph node involvement implies metastatic spread and a poor prognosis, lymph node involvement may be seen very early in KS and is of no special clinical significance. In fact, some patients may present with disease limited to the lymph nodes. These are generally patients with relatively intact immune function and thus the patients with the best prognosis. Pulmonary involvement with KS generally presents with shortness of breath. Some 80% of patients with pulmonary KS also have cutaneous lesions. The chest x-ray characteristically shows bilateral lower lobe infiltrates that obscure the margins of the mediastinum and diaphragm **(Fig. 197-43)**. Pleural effusions are seen in 70% of cases of pulmonary KS, a fact that is often helpful in the differential diagnosis. GI involvement is seen in 50% of patients with

FIGURE 197-42 Kaposi's sarcoma in three patients with AIDS demonstrating (***A***) periorbital edema and bruising; (***B***) classic truncal distribution of lesions; and (***C***) upper extremity lesions.

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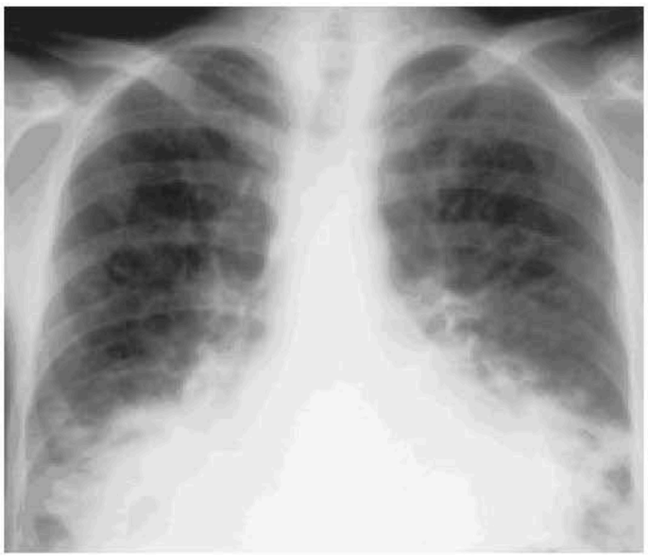

FIGURE 197-43 Chest x-ray of a patient with AIDS and pulmonary Kaposi's sarcoma. The characteristic findings include dense bilateral lower lobe infiltrates obscuring the heart borders and pleural effusions.

KS and usually takes one of two forms: (1) mucosal involvement, which may lead to bleeding that can be severe; these patients sometimes also develop symptoms of GI obstruction if lesions become large; and (2) biliary tract involvement. KS lesions may infiltrate the gallbladder and biliary tree, leading to a clinical picture of obstructive jaundice similar to that seen with sclerosing cholangitis. Several staging systems have been proposed for KS. One in common use was developed by the National Institute of Allergy and Infectious Diseases AIDS Clinical Trials Group; it distinguishes patients on the basis of tumor extent, immunologic function, and presence or absence of systemic disease (**Table 197-17**).

A diagnosis of KS is based on biopsy of a suspicious lesion. Histologically one sees a proliferation of spindle cells and endothelial cells, extravasation of red blood cells, hemosiderin-laden macrophages, and, in early cases, an inflammatory cell infiltrate. Included in the differential diagnosis are lymphoma (particularly for oral lesions), bacillary angiomatosis, and cutaneous mycobacterial infections.

Management of KS (**Table 197-18**) should be carried out in consultation with an expert since definitive treatment guidelines do not exist. In the majority of cases, effective cART will go a long way in achieving control. Antiretroviral therapy has been associated with the spontaneous regression of KS lesions. Paradoxically, it has also been associated with the initial appearance of KS as a form of IRIS. For patients in whom tumor persists or is compromising vital functions or in whom control of HIV replication is not possible, a variety of options exist. In some cases, lesions remain quite indolent, and many of these patients can be managed with no specific treatment. Fewer than 10% of AIDS patients with KS die as a consequence of their malignancy, and death from secondary infections is considerably more common. Thus, whenever possible one should avoid treatment regimens that may further suppress the immune system and increase susceptibility to opportunistic infections. Treatment is indicated under two main circumstances. The first is when a single lesion or a limited number of lesions are causing significant discomfort or cosmetic problems, such as with prominent facial lesions, lesions overlying a joint, or lesions in the oropharynx that interfere with swallowing or breathing. Under these circumstances, treatment with localized radiation, intralesional vinblastine, topical 9-*cis*-retinoic acid, or cryotherapy may be helpful. It should be noted that patients with HIV infection are particularly sensitive to the side effects of radiation therapy. This is especially true with respect to the development of radiation-induced mucositis; doses of radiation directed at mucosal surfaces, particularly in the head and neck region, should be adjusted accordingly. The use of systemic therapy, either IFN-α or chemotherapy, should be considered in patients with a large number of lesions or in patients with visceral involvement. The single most important determinant of response appears to be the CD4+ T cell count. This relationship between response rate and baseline CD4+ T cell count is particularly true for IFN-α. The response rate to IFN-α for patients with CD4+ T cell counts >600/μL is ~80%, while the response rate for patients with counts <150/μL is <10%. In contrast to the other systemic therapies, IFN-α provides an added advantage of having antiretroviral activity; thus, it may be the appropriate first choice for single-agent systemic therapy for early patients with disseminated disease. A variety of chemotherapeutic agents also have been shown to have activity against KS. Four of them—liposomal daunorubicin, liposomal doxorubicin, vinblastine, and paclitaxel—have been approved by the FDA for this indication. Liposomal daunorubicin is approved as first-line therapy for patients with advanced KS. It has fewer side effects than conventional chemotherapy. In contrast, liposomal doxorubicin and paclitaxel are approved only for KS patients who have failed standard chemotherapy. Response rates vary from 23% to 88%, appear to be comparable to what had been achieved earlier with combination chemotherapy regimens, and are greatly influenced by CD4+ T cell count.

Lymphomas occur with an increased frequency in patients with congenital or acquired T cell immunodeficiencies (**Chap. 344**). AIDS is no exception; at least 6% of all patients with AIDS develop lymphoma at some time during the course of their illness. This is a 120-fold increase in incidence compared with the general population. In contrast to the situation with KS, primary CNS lymphoma, and most opportunistic infections, the incidence of AIDS-associated systemic lymphomas has not experienced a dramatic decrease as a consequence of the widespread use of effective cART. Lymphoma occurs in all risk groups,

TABLE 197-17 National Institute of Allergy and Infectious Diseases AIDS Clinical Trials Group TIS Staging System For Kaposi's Sarcoma

PARAMETER	GOOD RISK (STAGE 0): ALL OF THE FOLLOWING	POOR RISK (STAGE 1): ANY OF THE FOLLOWING
Tumor (T)	Confined to skin and/or lymph nodes and/or minimal oral disease	Tumor-associated edema or ulceration Extensive oral lesions GI lesions Nonnodal visceral lesions
Immune system (I)	CD4+ T cell count ≥200/μL	CD4+ T cell count <200/μL
Systemic illness (S)	No B symptoms[a]	B symptoms[a] present
	Karnofsky performance status ≥70	Karnofsky performance status <70
	No history of opportunistic infection, neurologic disease, lymphoma, or thrush	History of opportunistic infection, neurologic disease, lymphoma, or thrush

[a]Defined as unexplained fever, night sweats, >10% involuntary weight loss, or diarrhea persisting for more than 2 weeks.

TABLE 197-18 Management of AIDS-Associated Kaposi's Sarcoma

Observation and optimization of antiretroviral therapy
Single or limited number of lesions
Radiation
Intralesional vinblastine
Cryotherapy
Extensive disease
Initial therapy
Interferon α (if CD4+ T cells >150/μL)
Liposomal daunorubicin
Subsequent therapy
Liposomal doxorubicin
Paclitaxel
Combination chemotherapy with low-dose doxorubicin, bleomycin, and vinblastine (ABV)
Targeted radiation

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with the highest incidence in patients with hemophilia and the lowest incidence in patients from the Caribbean or Africa with heterosexually acquired infection. Lymphoma is a late manifestation of HIV infection, generally occurring in patients with CD4+ T cell counts <200/μL. As HIV disease progresses, the risk of lymphoma increases. The attack rate for lymphoma increases exponentially with increasing duration of HIV infection and decreasing level of immunologic function. At 3 years following a diagnosis of HIV infection, the risk of lymphoma is 0.8% per year; by 8 years after infection, it is 2.6% per year. As individuals with HIV infection live longer as a consequence of improved cART and better treatment and prophylaxis of opportunistic infections, it is anticipated that the incidence of lymphomas may increase.

Three main categories of lymphoma are seen in patients with HIV infection: grade III or IV immunoblastic lymphoma, Burkitt's lymphoma, and primary CNS lymphoma. Approximately 90% of these lymphomas are B cell in phenotype; more than half contain EBV DNA. Some are associated with KSHV. These tumors may be either monoclonal or oligoclonal in nature and are probably in some way related to the pronounced polyclonal B cell activation seen in patients with AIDS.

Immunoblastic lymphomas account for ~60% of the cases of lymphoma in patients with AIDS. The majority of these are diffuse large B cell lymphomas (DLBCL). They are generally high grade and would have been classified as diffuse histiocytic lymphomas in earlier classification schemes. This tumor is more common in older patients, increasing in incidence from 0% in HIV-infected individuals <1 year old to >3% in those >50 years of age. Two variants of immunoblastic lymphoma that are seen primarily in HIV-infected patients are primary effusion lymphoma (PEL) and its solid variant, plasmacytic lymphoma of the oral cavity. PEL, also referred to as body cavity lymphoma, presents with lymphomatous pleural, pericardial, and/or peritoneal effusions in the absence of discrete nodal or extranodal masses. The tumor cells do not express surface markers for B cells or T cells and are felt to represent a preplasmacytic stage of differentiation. While both HHV-8 and EBV DNA sequences have been found in the genomes of the malignant cells from patients with body cavity lymphoma, KSHV is felt to be the driving force behind the oncogenesis (see above).

Small noncleaved cell lymphoma (*Burkitt's lymphoma*) accounts for ~20% of the cases of lymphoma in patients with AIDS. It is most frequent in patients 10–19 years old and usually demonstrates characteristic c-*myc* translocations from chromosome 8 to chromosome 14 or 22. Burkitt's lymphoma is not commonly seen in the setting of immunodeficiency other than HIV-associated immunodeficiency, and the incidence of this particular tumor is more than 1000-fold higher in the setting of HIV infection than in the general population. In contrast to African Burkitt's lymphoma, where 97% of the cases contain EBV genome, only 50% of HIV-associated Burkitt's lymphomas are EBV-positive.

Primary CNS lymphoma accounts for ~20% of the cases of lymphoma in patients with HIV infection. In contrast to HIV-associated Burkitt's lymphoma, primary CNS lymphomas are usually positive for EBV. In one study, the incidence of Epstein-Barr positivity was 100%. This malignancy does not have a predilection for any particular age group. The median CD4+ T cell count at the time of diagnosis is ~50/μL. Thus, CNS lymphoma generally presents at a later stage of HIV infection than does systemic lymphoma. This may explain, at least in part, the poorer prognosis for this subset of patients.

The clinical presentation of lymphoma in patients with HIV infection is quite varied, ranging from focal seizures to rapidly growing mass lesions in the oral mucosa (**Fig. 197-44**) to persistent unexplained fever. At least 80% of patients present with extranodal disease, and a similar percentage have B-type symptoms of fever, night sweats, or weight loss. Virtually any site in the body may be involved. The most common extranodal site is the CNS, which is involved in approximately one-third of all patients with lymphoma. Approximately 60% of these cases are primary CNS lymphoma. Primary CNS lymphoma generally presents with focal neurologic deficits, including cranial nerve findings, headaches, and/or seizures. MRI or CT generally reveals a limited number (one to three) of 3- to 5-cm lesions (**Fig. 197-45**). The lesions often show ring enhancement on contrast administration and may occur in any location. Contrast enhancement is usually less pronounced than that seen with toxoplasmosis. Locations that are most commonly involved with CNS lymphoma are deep in the white matter. The main diseases in the differential diagnosis are cerebral toxoplasmosis and cerebral Chagas' disease. In addition to the 20% of lymphomas in HIV-infected individuals that are primary CNS lymphomas, CNS disease is also seen in HIV-infected patients with systemic lymphoma. Approximately 20% of patients with systemic lymphoma have CNS disease in the form of leptomeningeal involvement. This fact underscores the importance of lumbar puncture in the staging evaluation of patients with systemic lymphoma.

FIGURE 197-44 Immunoblastic lymphoma involving the hard palate of a patient with AIDS.

Systemic lymphoma is seen at earlier stages of HIV infection than primary CNS lymphoma. In one series the mean CD4+ T cell count was 226/μL. In addition to lymph node involvement, systemic lymphoma may commonly involve the GI tract, bone marrow, liver, and lung. GI tract involvement is seen in ~25% of patients. Any site in the GI tract may be involved, and patients may complain of difficulty swallowing or abdominal pain. The diagnosis is usually suspected on the basis of CT or MRI of the abdomen. Bone marrow involvement is seen in ~20% of patients and may lead to pancytopenia. Liver and lung involvement are each seen in ~10% of patients. Pulmonary disease may present as a mass lesion, multiple nodules, or an interstitial infiltrate.

Both conventional and unconventional approaches have been employed in an attempt to treat HIV-related lymphomas. Systemic lymphoma is generally treated by the oncologist with combination chemotherapy. Earlier disappointing figures are being replaced with

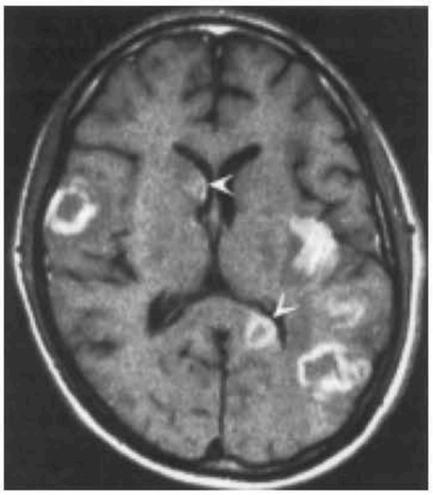

FIGURE 197-45 Central nervous system lymphoma. Postcontrast T1-weighted MRI scan in a patient with AIDS, altered mental status, and hemiparesis. Multiple enhancing lesions, some ring-enhancing, are present. The left sylvian lesion shows gyral and subcortical enhancement, and the lesions in the caudate and splenium (*arrowheads*) show enhancement of adjacent ependymal surfaces.

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more optimistic results for the treatment of systemic lymphoma following the availability of more effective cART and the use of rituximab in CD20+ tumors. While there is some controversy regarding the use of antiretrovirals during chemotherapy, there is no question that their use overall in patients with HIV lymphoma has improved survival. Concerns regarding synergistic bone marrow toxicities with chemotherapy and cART are mitigated with the use of cART regimens that avoid bone marrow–toxic antiretrovirals. As in most situations in patients with HIV disease, those with higher CD4+ T cell counts tend to fare better. Response rates as high as 72% with a median survival of 33 months and disease-free intervals up to 9 years have been reported. Treatment of primary CNS lymphoma remains a significant challenge. Treatment is complicated by the fact that this illness usually occurs in patients with advanced HIV disease. Palliative measures such as radiation therapy provide some relief. The prognosis remains poor in this group, with a 2-year survival of 29%.

Multicentric Castleman's disease is a KSHV-associated lymphoproliferative disorder that is seen with an increased frequency in patients with HIV infection. While not a true malignancy, it shares many features with lymphoma including generalized lymphadenopathy, hepatosplenomegaly, and systemic symptoms of fever, fatigue, and weight loss. Pulmonary symptoms may be seen in ~50% of patients. KS is present in 75–82% of cases. Lymph node biopsies reveal a predominance of interfollicular plasma cells and/or germinal centers with vascularization and an "onionskin" (hyaline vascular) appearance. Prior to the availability of cART, HIV-infected patients with multicentric Castleman's disease had a 15-fold increased risk of developing non-Hodgkin's lymphoma compared with HIV-infected patients in general. Treatment typically involves chemotherapy. Anecdotal reports of success with rituximab suggest that more specific treatment may be successful, although, in one series treatment with rituximab was associated with worsening of coexisting KS. The median survival of patients with treated multicentric Castleman's disease pre-cART was initially reported as 14 months. This has increased to a 2-year survival of more than 90% in the era of cART.

Evidence of infection with *human papillomavirus* (HPV), associated with *intraepithelial dysplasia of the cervix* or *anus*, is approximately twice as common in HIV-infected individuals as in the general population and can lead to intraepithelial neoplasia and eventually invasive cancer. In a series of studies, HIV-infected men were examined for evidence of anal dysplasia, and Papanicolaou (Pap) smears were found to be abnormal in 20–80%. These changes tend to persist and are generally not affected by cART, raising the possibility of a subsequent transition to a more malignant condition. While the incidence of an abnormal Pap smear of the cervix is ~5% in otherwise healthy women, the incidence of abnormal cervical smears in women with HIV infection is 30–60%, and *invasive cervical cancer* is included as an AIDS-defining condition. While only small increases in the absolute numbers of cervical or anal cancers have been seen as a consequence of HIV infection, the relative risk of these conditions when one compares HIV-infected to noninfected men and women is on the order of 10- to 100-fold. Given the high rates of dysplasia and relative risks for cervical and anal cancer, a comprehensive gynecologic and rectal examination, including Pap smear, is indicated at the initial evaluation and 6 months later for all patients with HIV infection. If these examinations are negative at both time points, the patient should be followed with yearly evaluations. If an initial or repeat Pap smear shows evidence of severe inflammation with reactive squamous changes, the next Pap smear should be performed at 3 months. If, at any time, a Pap smear shows evidence of squamous intraepithelial lesions, colposcopic examination with biopsies as indicated should be performed. The 2-year survival rate for HIV-infected patients with invasive cervical cancer is 64% compared with 79% in non-HIV-infected patients. In addition to rectal and cervical lesions, HPV can also lead to head and neck cancers. In one study of men who have sex with men, 25% were found to have oral HPV; high-risk HPV genotypes were three times more common in the HIV-infected men. The most common HPV genotypes in the general population and the genotypes upon which current HPV vaccines are based are 6, 11, 16, and 18. This is not the case in the HIV-infected population, where other genotypes such as 58 and 53 also are prominent. This raises concerns about the level of effectiveness of the current HPV vaccines for HIV-infected patients. Despite this, it is recommended that patients with HIV infection be vaccinated against HPV.

IDIOPATHIC CD4+ T LYMPHOCYTOPENIA

A syndrome was recognized in 1992 characterized by an absolute CD4+ T cell count of <300/μL or <20% of total T cells on a minimum of two occasions at least 6 weeks apart; no evidence of HIV-1, HIV-2, HTLV-1, or HTLV-2 on testing; and the absence of any defined immunodeficiency or therapy associated with decreased levels of CD4+ T cells. By mid-1993, ~100 patients had been described. After extensive multicenter investigations, a series of reports were published in early 1993, which together allowed a number of conclusions. Idiopathic CD4+ lymphocytopenia (ICL) is a very rare syndrome, as determined by studies of blood donors and cohorts of HIV-seronegative men who have sex with men. Cases were clearly identified as early as 1983 and were remarkably similar to the clinical features of ICL that had been identified decades earlier. The definition of ICL based on CD4+ T cell counts coincided with the ready availability of testing for CD4+ T cells in patients suspected of being immunodeficient. Although, as a result of immune deficiency, certain patients with ICL develop some of the opportunistic diseases (particularly cryptococcosis, nontuberculous mycobacterial infections, and cervical dysplasia) seen in HIV-infected patients, the syndrome is demographically, clinically, and immunologically unlike HIV infection and AIDS. Fewer than half of the reported ICL patients had risk factors for HIV infection, and there were wide geographic and age distributions. The fact that a significant proportion of patients did have risk factors probably reflects a selection bias, in that physicians who take care of HIV-infected patients are more likely to monitor CD4+ T cells. Approximately half of the patients are women, compared with approximately one-third among HIV-infected individuals in the United States. Many patients with ICL remained clinically stable, and their condition did not deteriorate progressively as is common with seriously immunodeficient HIV-infected patients. Approximately 15% of patients with ICL experience spontaneous reversal of the CD4+ T lymphocytopenia. Immunologic abnormalities in ICL are somewhat different from those of HIV infection. ICL patients often have increases in CD4+ T cell activation with decreases in CD8+ T cells and B cells. Furthermore, immunoglobulin levels are either normal or, more commonly, decreased in patients with ICL, compared with the usual hypergammaglobulinemia of HIV-infected individuals. Virologic studies of these patients have revealed no evidence of HIV-1, HIV-2, HTLV-1, or HTLV-2 or of any other mononuclear cell–tropic virus. Furthermore, there has been no epidemiologic evidence to suggest that a transmissible microbe was involved. The cases of ICL have been widely dispersed, with no clustering. Close contacts and sexual partners who were studied were clinically well and were serologically, immunologically, and virologically negative for HIV. ICL is a heterogeneous syndrome, and it is highly likely that there is no common cause; however, there may be common causes among subgroups of patients that are currently unrecognized.

Patients who present with laboratory data consistent with ICL should be worked up for underlying diseases that could be responsible for the immune deficiency. If no underlying cause is detected, no specific therapy should be initiated. However, if opportunistic diseases occur, they should be treated appropriately (see above). Depending on the level of the CD4+ T cell count, patients should receive prophylaxis for the commonly encountered opportunistic infections.

TREATMENT

AIDS and Related Disorders

GENERAL PRINCIPLES OF PATIENT MANAGEMENT

The CDC guidelines call for the testing for HIV infection to be a part of routine medical care. It is recommended that the patient be informed of the intention to test, as is the case with other routine laboratory determinations, and be given the opportunity to "opt

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out." Such an approach is critical to the goal of identifying as many infected individuals as possible since 15% of the 1.1 million individuals in the United States who are HIV-infected are not aware of their status. Under these circumstances of routine testing, although it is desirable, pretest counseling may not always be built into the testing process. However, no matter how well prepared a patient is for adversity, the discovery of a diagnosis of HIV infection is a devastating event. Thus, physicians should be sensitive to this fact and, where possible, execute some degree of pretest counseling to at least partially prepare the patient should the results demonstrate the presence of HIV infection. Following a diagnosis of HIV infection, the health care provider should be prepared to immediately activate support systems for the newly diagnosed patient and initiate cART therapy. These supports should include individuals who can spend time talking to the newly diagnosed person and ensuring that he or she is emotionally stable and ready to begin therapy. Most communities have HIV support centers that can be of great help in these difficult situations.

The treatment of patients with HIV infection requires not only a comprehensive knowledge of the possible disease processes that may occur and up-to-date knowledge of and experience with cART, but also the ability to deal with the problems of a chronic, potentially life-threatening illness. A comprehensive knowledge of internal medicine is required to deal with the changing spectrum of illnesses associated with HIV infection, many of which are similar to a state of accelerated aging. Great advances have been made in the treatment of patients with HIV infection. The appropriate use of potent cART and other treatment and prophylactic interventions are of critical importance in providing each patient with the best opportunity to live a long and healthy life with HIV infection. In contrast to the earlier days of this epidemic, a diagnosis of HIV infection need no longer be equated with having an inevitably fatal disease. In addition to medical interventions, the health care provider has a responsibility to provide each patient with appropriate counseling and education concerning their disease as part of a comprehensive care plan. Patients must be educated about the potential transmissibility of their infection and about the fact that while health care providers may refer to levels of the virus as "undetectable," this is only a reflection of the sensitivity of the assay being used to measure the virus, rather than a comment on the presence or absence of the virus. It is important for patients to be aware that the virus is still present in virtually all patients who have ever been diagnosed with HIV infection and capable of being transmitted at all stages of HIV disease. Thus, there must be frank discussions concerning sexual practices and the sharing of syringes and other paraphernalia used in illicit drug use. The treating physician not only must be aware of the latest medications available for patients with HIV infection but also must educate patients concerning the natural history of their illness and listen and be sensitive to their fears and concerns. As with other diseases, therapeutic decisions should be made in consultation with the patient, when possible, and with the patient's proxy if the patient is incapable of making decisions. In this regard, it is recommended that all patients with HIV infection, and in particular those with CD4+ T cell counts <200/μL, designate a trusted individual with durable power of attorney to make medical decisions on their behalf, if necessary.

Following a diagnosis of HIV infection, there are several examinations and laboratory studies that should be performed to help determine the extent of disease and provide baseline standards for future reference (**Table 197-19**). In addition to routine chemistry, fasting lipid profile, aspartate aminotransferase, alanine aminotransferase, total and direct bilirubin, fasting glucose and hematology screening panels, Pap smear, urinalysis, and chest x-ray, one should also obtain a CD4+ T cell count, a plasma HIV RNA level, an HIV resistance test, a rapid plasma reagin or VDRL test, an anti-*Toxoplasma* antibody titer, and serologies for hepatitis A, B, and C. A PPD test or IFN-γ release assay should be done and an MMSE performed and recorded. A pregnancy test should be done in women in whom the drug efavirenz is being considered, and HLA-B5701 testing should be done in all patients in whom the drug abacavir is being considered. Patients should be immunized with pneumococcal polysaccharide, with annual influenza shots, and, if seronegative for these viruses, with HPV, hepatitis A, and hepatitis B vaccines. The status of hepatitis C infection should be determined. In addition, patients should be counseled with regard to sexual practices and needle sharing, and counseling should be offered to those whom the patient knows or suspects may also be infected. Once these baseline activities are performed, short- and long-term medical management strategies should be developed based on the most recent information available and modified as new information becomes available. The field of HIV medicine is changing rapidly, and it is difficult to remain fully up to date. Fortunately there are a series of excellent sites on the Internet that are frequently updated, and they provide the most recent information on a variety of topics, including consensus panel reports on treatment (**Table 197-20**).

TABLE 197-19 Initial Evaluation of the Patient with HIV Infection

History and physical examination
Routine chemistry and hematology
AST, ALT, alkaline phosphatase, direct and indirect bilirubin
Lipid profile and fasting glucose
CD4+ T lymphocyte count
Plasma HIV RNA level
HIV resistance testing
HLA-B5701 screening
RPR or VDRL test
Anti-*Toxoplasma* antibody titer
Urinalysis
PPD skin test or IFN-γ release assay
Mini-Mental Status Examination
Serologies for hepatitis A, hepatitis B, and hepatitis C
Immunization with pneumococcal polysaccharide; influenza; HPV as indicated
Immunization with hepatitis A and hepatitis B if seronegative
Counseling regarding natural history and transmission
Help contacting others who might be infected

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; PPD, purified protein derivative; RPR, rapid plasma reagin; VDRL, Venereal Disease Research Laboratory.

ANTIRETROVIRAL THERAPY

Combination antiretroviral therapy (cART), also referred to as highly active antiretroviral therapy (HAART), is the cornerstone of management of patients with HIV infection. Following the initiation of widespread use of cART in the United States in 1995–1996, marked declines were noted in the incidence of most AIDS-defining conditions (Fig. 197-34). Suppression of HIV replication is an important component in prolonging life as well as in improving the quality of life in patients with HIV infection. Adequate suppression requires strict adherence to prescribed regimens of antiretroviral drugs. This has been facilitated by the coformulations of antiretrovirals and the development of once-daily regimens. Unfortunately, many of the most important questions related to the treatment of HIV disease currently lack definitive answers. Among the decisions that need to be made in the context of prescribing cART are when therapy should be started, selection of the best initial regimen, when a given regimen should be changed, and what it

TABLE 197-20 HIV Disease Resources Available on the World Wide Web

www.aidsinfo.nih.gov	AIDSinfo, a service of the U.S. Department of Health and Human Services, posts federally approved treatment guidelines for HIV and AIDS; provides information on federally funded and privately funded clinical trials and CDC publications and data
www.cdcnpin.org	Updates on epidemiologic data and prevention information from the CDC

Abbreviation: CDC, Centers for Disease Control and Prevention.

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should be changed to when a change is made. The care provider and patient must come to a mutually agreeable plan based on the best available data. In an effort to facilitate this process, the U.S. Department of Health and Human Services makes available on the Internet (*www.aidsinfo.nih.gov*) a series of periodically updated guidelines, including *"Guidelines for the Use of Antiretroviral Agents in HIV-Infected Adults and Adolescents"* and *"Guidelines for the Prevention of Opportunistic Infections in Persons Infected with Human Immunodeficiency Virus."* At present, an extensive clinical trials network, involving both clinical investigators and patient advocates, is in place attempting to develop improved approaches to therapy. Consortia comprising representatives of academia, industry, independent foundations, and the federal government are involved in the process of drug development, including a wide-ranging series of clinical trials. As a result, new therapies and new therapeutic strategies are continually emerging. New drugs are often available through expanded-access programs prior to official licensure. Given the complexity of this field, decisions regarding cART are best made in consultation with experts.

Currently available drugs for the treatment of HIV infection as part of a combination regimen fall into four categories: those that inhibit the viral reverse transcriptase enzyme (nucleoside and nucleotide reverse transcriptase inhibitors; nonnucleoside reverse transcriptase inhibitors), those that inhibit the viral protease enzyme (protease inhibitors), those that inhibit the viral integrase enzyme (integrase inhibitors), and those that interfere with viral entry (fusion inhibitors; CCR5 antagonists) (**Table 197-21; Fig. 197-46**). Numerous formulations combining two or more of these antiretroviral drugs have been licensed (**Table 197-22**). Prior to initiation of therapy and at any time a change in therapy due to treatment failure is being considered, drug resistance testing should be performed to help guide the selection of drugs to be used in combination. A summary of known resistance mutations for antiretroviral drugs is shown in **Fig. 197-47**.

The FDA-approved reverse transcriptase inhibitors include the *nucleoside analogues* zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, and emtricitabine; the *nucleotide analogues* tenofovir disoproxil and tenofovir alafenamide; and the *nonnucleoside reverse transcriptase inhibitors* nevirapine, delavirdine, efavirenz, etravirine, and rilpivirine (Table 197-21). These represent the first class of drugs licensed for the treatment of HIV infection. They are indicated for this use as part of combination regimens. It should be stressed that none of these drugs should be used as monotherapy for HIV infection due to the relative ease with which drug resistance may develop under such circumstances. Thus, when lamivudine, emtricitabine, or tenofovir is used to treat hepatitis B infection in the setting of HIV infection, one should ensure that the patient is also on additional antiretroviral medication. The reverse transcriptase inhibitors block the HIV replication cycle at the point of RNA-dependent DNA synthesis, the reverse transcription step. While the nonnucleoside reverse transcriptase inhibitors are quite selective for the HIV-1 reverse transcriptase, the nucleoside and nucleotide analogues inhibit a variety of DNA polymerases in addition to those of the HIV-1 reverse transcriptase. For this reason, serious side effects are more varied with the nucleoside analogues and include mitochondrial damage that can lead to hepatic steatosis and lactic acidosis as well as peripheral neuropathy and pancreatitis. The use of either of the thymidine analogues zidovudine and stavudine has been associated with a syndrome of hyperlipidemia, glucose intolerance/insulin resistance, and fat redistribution often referred to as *lipodystrophy syndrome* (discussed in "Diseases of the Endocrine System and Metabolic Disorders," above). The reverse transcriptase inhibitors preferred for use in combination regimens according to the DHHS Panel on the use of antiretroviral drugs are lamivudine, emtricitabine, abacavir, tenofovir disoproxil, and tenofovir alafenamide.

The HIV-1 protease inhibitors (saquinavir, indinavir, ritonavir, nelfinavir, amprenavir, fosamprenavir, lopinavir/ritonavir, atazanavir, tipranavir, and darunavir) are an important part of the therapeutic armamentarium of antiretrovirals. While possessing antiviral properties of its own, ritonavir is typically used as a pharmacokinetic enhancer due to its high affinity for several isoforms of cytochrome P450 (3A4, 2D6) leading to large increases in the plasma concentrations of co-administered drugs metabolized by these pathways As in the case of reverse transcriptase inhibitors, resistance to protease inhibitors can develop rapidly in the setting of monotherapy, and thus these agents should be used only as part of combination therapeutic regimens. Based upon superior efficacy and side-effect profile, ritonavir-boosted darunavir in combination with emtricitabine and tenofovir disoproxil or tenofovir alafenamide is the protease inhibitor strategy preferred for initial therapy according to the DHHS Panel on the use of antiretroviral drugs.

Integrase inhibitors act by blocking the action of the HIV integrase enzyme and thus preventing integration of the HIV provirus into the host cell genome. They are among the most potent and safest of the antiretroviral drugs and frequently part of initial combination regimens. The four licensed integrase inhibitors are raltegravir, elvitegravir, dolutegravir, and bictegravir. Elvitegravir is always given in combination with cobicistat, which acts to boost the concentrations of elvitegravir. Cobicistat also inhibits tubular secretion of creatinine, resulting in increases in serum creatinine, and is not recommended for patients with estimated creatinine clearances <70 mL/min. Bictegravir is available only in combination with tenofovir alafenamide and emtricitabine.

TABLE 197-21 Antiretroviral Drugs Most Commonly Used in the Treatment of HIV Infection

DRUG	STATUS	INDICATION	DOSE IN COMBINATION	SUPPORTING DATA	TOXICITY
Nucleoside or Nucleotide Reverse Transcriptase Inhibitors					
Zidovudine (AZT, azidothymidine, *Retrovir, 3'azido-3'-deoxythymidine)	Licensed	Treatment of HIV infection in combination with other antiretroviral agents	200 mg q8h or 300 mg bid	19 vs 1 death in original placebo-controlled trial in 281 patients with AIDS or ARC	Anemia, granulocytopenia, myopathy, lactic acidosis, hepatomegaly with steatosis, headache, nausea, nail pigmentation, lipid abnormalities, lipoatrophy, hyperglycemia
		Prevention of maternal-fetal HIV transmission		In pregnant women with CD4+ T cell count ≥200/μL, AZT PO beginning at weeks 14–34 of gestation plus IV drug during labor and delivery plus PO AZT to infant for 6 weeks decreased transmission of HIV by 67.5% (from 25.5% to 8.3%); n = 363	

(*Continued*)

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TABLE 197-21 Antiretroviral Drugs Most Commonly Used in the Treatment of HIV Infection (Continued)

DRUG	STATUS	INDICATION	DOSE IN COMBINATION	SUPPORTING DATA	TOXICITY
Lamivudine (Epivir, 2'3'-dideoxy-3'-thiacytidine, 3TC)	Licensed	In combination with other antiretroviral agents for the treatment of HIV infection	150 mg bid 300 mg qd	In combination with AZT superior to AZT alone with respect to changes in CD4+ T cell counts in 495 patients who were zidovudine-naïve and 477 patients who were zidovudine-experienced; overall CD4+ T cell counts for the zidovudine group were at baseline by 24 weeks, while in the group treated with zidovudine plus lamivudine, they were 10–50 cells/μL above baseline; 54% decrease in progression to AIDS/death compared with AZT alone	Flare of hepatitis in HBV-co-infected patients who discontinue drug
Emtricitabine (FTC, Emtriva)	Licensed	In combination with other antiretroviral agents for the treatment of HIV infection	200 mg qd	~~Comparable to stavudine in combination with didanosine and efavirenz in 571 treatment-naïve patients; similar to 3TC in combination with AZT or stavudine + NNRTI or PI in 440 patients doing well for ≥12 weeks on a 3TC regimen~~	Hepatotoxicity in HBV-co-infected patients who discontinue drug, skin discoloration
Abacavir (Ziagen)	Licensed	For treatment of HIV infection in combination with other antiretroviral agents	300 mg bid	Abacavir + AZT + 3TC equivalent to indinavir + AZT + 3TC with regard to viral load suppression (~60% in each group with <400 HIV RNA copies/mL plasma) and CD4+ T cell increase (~100/μL in each group) at 24 weeks	Hypersensitivity reaction In HLA-B5701+ individuals (can be fatal); fever, rash, nausea, vomiting, malaise or fatigue, and loss of appetite
Tenofovir disoproxil fumarate (Viread)	Licensed	For use in combination with other antiretroviral agents when treatment is indicated	300 mg qd	Reduction of ~0.6 log in HIV-1 RNA levels when added to background regimen in treatment-experienced patients	Renal, osteomalacia, flare of hepatitis in HBV-co-infected patients who discontinue drug
Tenofovir alafenamide (Vemlidy)	Licensed	In combination with emtricitabine and other antiretroviral agents for treatment of HIV-1 infection	25 mg qd	92% of patients treated in combination with emtricitabine, elvitegravir, and cobicistat had HIV-1 RNA levels <50 copies/mL	Nausea, less renal toxicity than tenofovir disoproxil fumarate
Non-Nucleoside Reverse Transcriptase Inhibitors					
Nevirapine (Viramune)	Licensed	In combination with other antiretroviral agents for treatment of progressive HIV infection	200 mg/d × 14 days then 200 mg bid *or* 400 mg extended release qd	Increase in CD4+ T cell count, decrease in HIV RNA when used in combination with nucleosides	Skin rash, hepatotoxicity
Efavirenz (Sustiva)	Licensed	For treatment of HIV infection in combination with other antiretroviral agents	600 mg qhs	Efavirenz + AZT + 3TC comparable to indinavir + AZT + 3TC with regard to viral load suppression (a higher percentage of the efavirenz group achieved viral load <50 copies/mL, but the discontinuation rate in the indinavir group was unexpectedly high, accounting for most treatment "failures"); CD4 cell increase (~140/μL in each group) at 24 weeks	Rash, dysphoria, elevated liver function tests, drowsiness, abnormal dreams, depression, lipid abnormalities, potentially teratogenic
Etravirine (Intelence)	Licensed	In combination with other antiretroviral agents in treatment-experienced patients whose HIV is resistant to nonnucleoside reverse transcriptase inhibitors and other antiretroviral medications	200 mg bid	Higher rates of HIV RNA suppression to <50 copies/mL (56% vs 39%); greater increases in CD4+ T cell count (89 vs 64 cells) compared to placebo when given in combination with an optimized background regimen	Rash, nausea, hypersensitivity reactions
Rilpivirine (Edurant)	Licensed	In combination with other drugs in previously untreated patients when treatment is indicated.	25 mg qd	Noninferior to efavirenz with respect to suppression at week 48 in 1368 treatment-naive individuals, except in patients with pretherapy HIV RNA levels >100,000 where it was inferior	Nausea, dizziness, somnolence, vertigo, less CNS toxicity and rash than efavirenz
Protease Inhibitors					
Ritonavir (Norvir)	Licensed	In combination with other antiretroviral agents for treatment of HIV infection when treatment is warranted	600 mg bid (also used in lower doses as pharmacokinetic booster)	Reduction in the cumulative incidence of clinical progression or death from 34% to 17% in patients with CD4+ T cell count <100/μL treated for a median of 6 months	Nausea, abdominal pain, hyperglycemia, fat redistribution, lipid abnormalities, may alter levels of many other drugs, paresthesias, hepatitis

(Continued)

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HHS-NIAID-20-0593-A-000949

TABLE 197-21 Antiretroviral Drugs Most Commonly Used in the Treatment of HIV Infection (Continued)

DRUG	STATUS	INDICATION	DOSE IN COMBINATION	SUPPORTING DATA	TOXICITY
Atazanavir (Reyataz)	Licensed	For treatment of HIV infection in combination with other antiretroviral agents	400 mg qd or 300 mg qd + ritonavir 100 mg qd when given with efavirenz	Comparable to efavirenz when given in combination with AZT + 3TC in a study of 810 treatment-naïve patients; comparable to nelfinavir when given in combination with stavudine + 3TC in a study of 467 treatment-naïve patients	Hyperbilirubinemia, PR prolongation, nausea, vomiting, hyperglycemia, fat maldistribution, rash transaminase elevations, renal stones
Darunavir (Prezista)	Licensed	In combination with 100 mg ritonavir for combination therapy in treatment-experienced adults	600 mg + 100 mg ritonavir twice daily with food	At 24 weeks, patients with prior extensive exposure to antiretrovirals treated with a new combination including darunavir showed a –1.89-log change in HIV RNA levels and a 92-cell increase in CD4+ T cells compared with –0.48 log and 17 cells in the control arm	Diarrhea, nausea, headache, skin rash, hepatotoxicity, hyperlipidemia, hyperglycemia
Entry Inhibitors					
Enfuvirtide (Fuzeon)	Licensed	In combination with other agents in treatment-experienced patients with evidence of HIV-1 replication despite ongoing anti-retroviral therapy	90 mg SC bid	In treatment of experienced patients, superior to placebo when added to new optimized background (37% vs 16% with <400 HIV RNA copies/mL at 24 weeks; + 71 vs + 35 CD4+ T cells at 24 weeks)	Local injection reactions, hypersensitivity reactions, increased rate of bacterial pneumonia
Maraviroc (Selzentry)	Licensed	In combination with other antiretroviral agents in adults infected with only CCR5-tropic HIV-1	150–600 mg bid depending on concomitant medications (see text)	At 24 weeks, among 635 patients with CCR5-tropic virus and HIV-1 RNA >5000 copies/mL despite at least 6 months of prior therapy with at least 1 agent from 3 of the 4 antiretroviral drug classes, 61% of patients randomized to maraviroc achieved HIV RNA levels <400 copies/mL compared with 28% of patients randomized to placebo	Hepatotoxicity, nasopharyngitis, fever, cough, rash, abdominal pain, dizziness, musculoskeletal symptoms
Ibalizumab (Trogarzo)	Licensed	In combination with other antiretroviral agents in patients with multidrug-resistant HIV-1	Single loading dose of 2000 mg followed by a maintenance dose of 800 mg every 2 weeks	At 25 weeks, 50% of patients with multi-drug resistant HIV-1 with HIV-1 RNA >1000 copies/mL treated with an optimized background of 1 active drug and ibalizumab achieved HIV RNA levels <200 copies/mL	Rash, diarrhea, nausea
Integrase Inhibitor					
Raltegravir (Isentress)	Licensed	In combination with other antiretroviral agents	400 mg bid	At 24 weeks, among 436 patients with 3-class drug resistance, 76% of patients randomized to receive raltegravir achieved HIV RNA levels <400 copies/mL compared with 41% of patients randomized to receive placebo	Nausea, headache, diarrhea, CPK elevation, muscle weakness, rhabdomyolysis
Elvitegravir (Available only in combination with cobicistat, tenofovir, and emtricitabine [Stribild])	Licensed	Fixed-dose combination	1 tablet daily	Noninferior to raltegravir or atazanavir/ritonavir in treatment-experienced patients.	Diarrhea, nausea, upper respiratory infections, headache
Dolutegravir (Tivicay)	Licensed	In combination with other antiretroviral agents	50 mg daily for treatment-naïve patients 50 mg twice daily for treatment-experienced patients or those also receiving efavirenz or rifampin	Noninferior to raltegravir, superior to efavirenz or darunavir/ritonavir	Insomnia, headache, hypersensitivity reactions, hepatotoxicity
Bictegravir (Available only in combination with tenofovir alafenamide and emtricitabine [Biktarvy])	Licensed	For treatment of HIV infection in adults	50 mg bictegravir/ 25 mg tenofovir alafenamide/ 200 mg emtricitabine qd	Non-inferior to dolutegravir/tenofovir/ emtricitabine and non-inferior to dolutegravir/abacavir/lamivudine	Nausea, diarrhea, headache

*Initial trade names are provided. Generic forms may be available.

Abbreviations: ARC, AIDS-related complex; NRTIs, nonnucleoside reverse transcriptase inhibitors.

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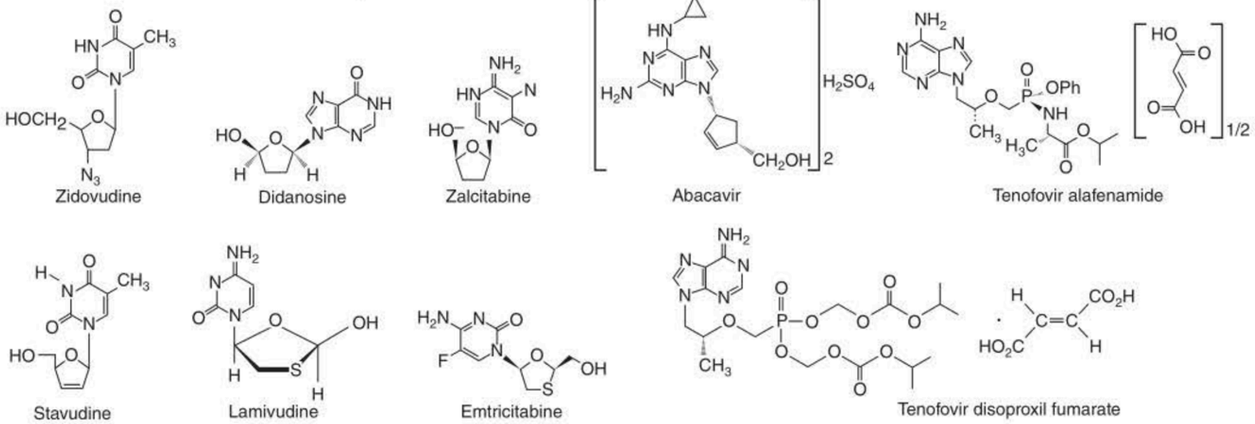
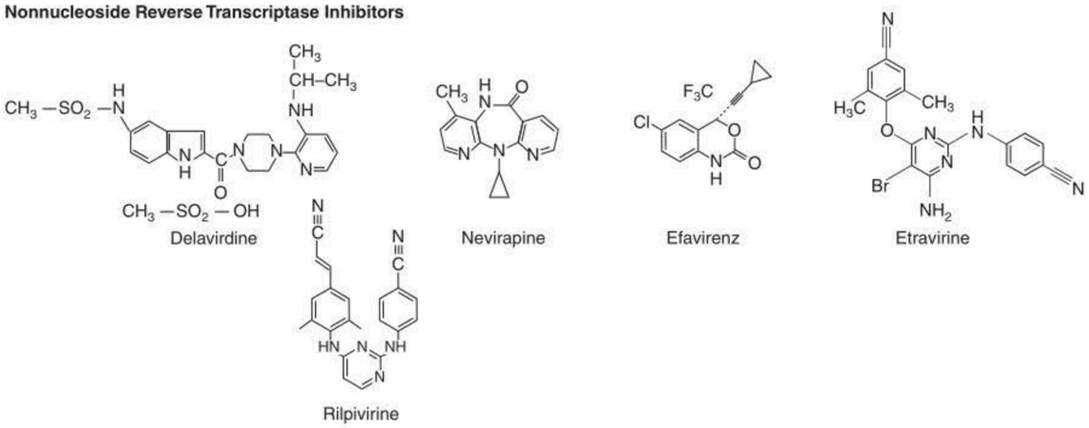
Nucleoside or Nucleotide Reverse Transcriptase Inhibitors



Nonnucleoside Reverse Transcriptase Inhibitors



Protease Inhibitors

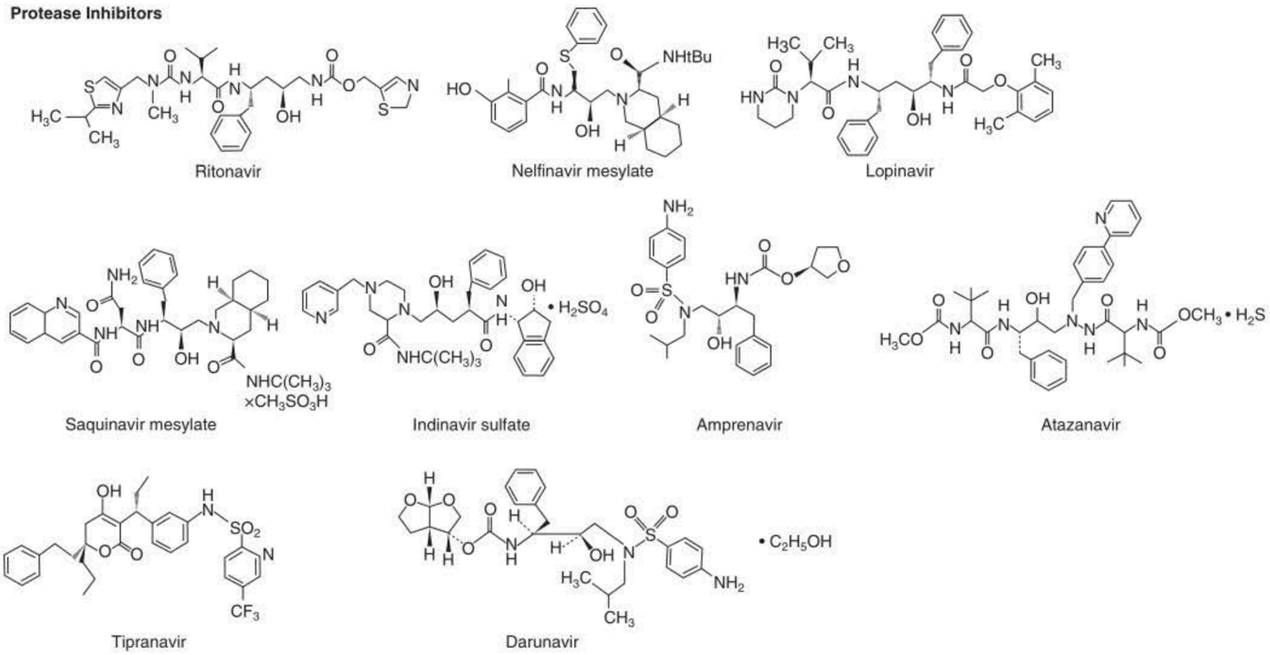


FIGURE 197-46 Molecular structures of antiretroviral agents.

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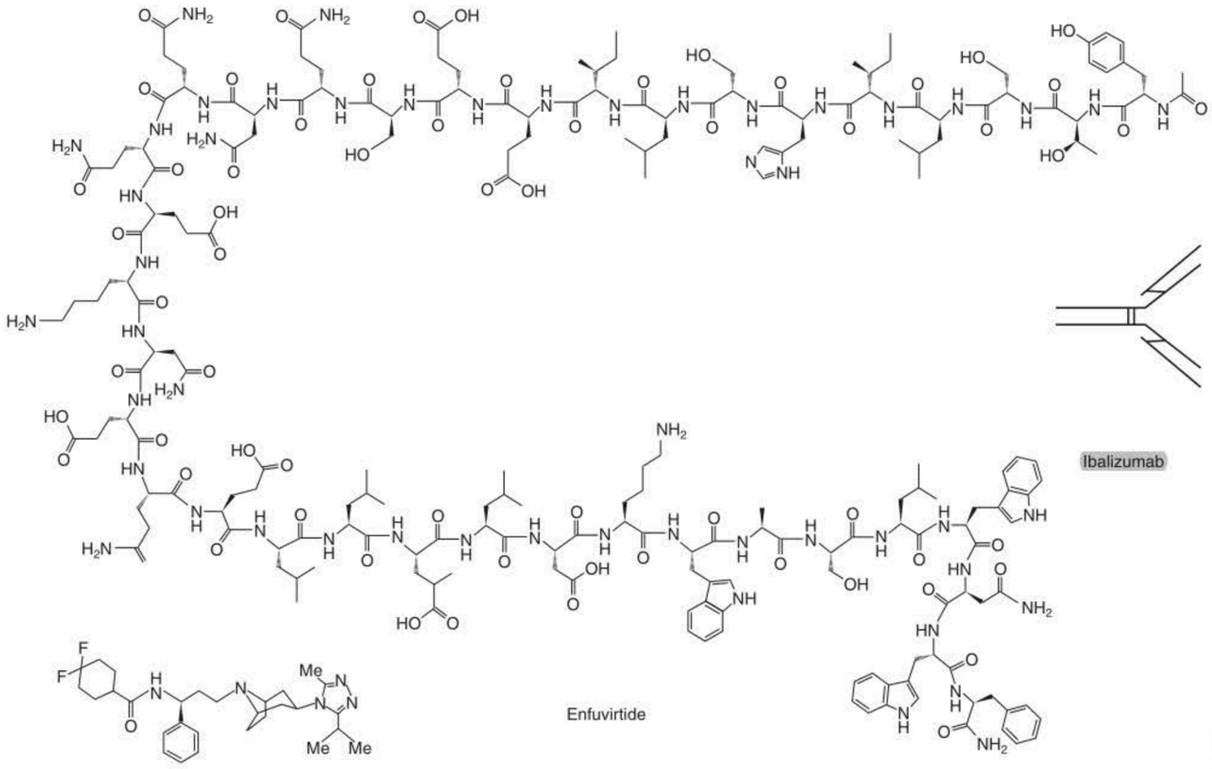

Integrase Inhibitors

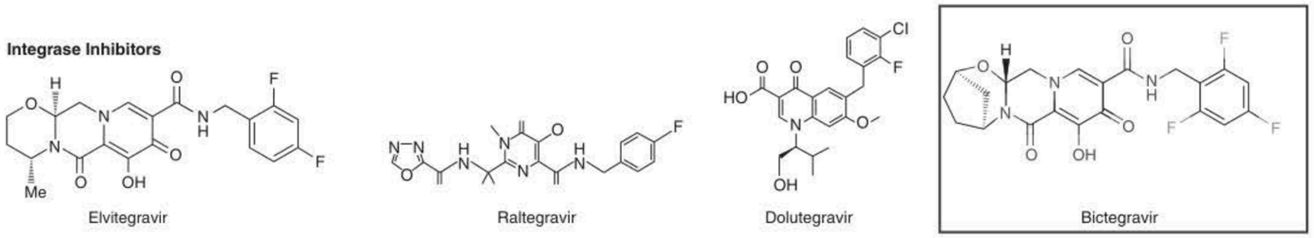

FIGURE 197-46 (*Continued*)

Entry inhibitors act by interfering with the binding of HIV to its receptor or co-receptor or by interfering with the process of fusion (see above). The first drug in this class to be licensed was the fusion inhibitor *enfuvirtide*, or T-20, followed by the CCR5 antagonist *maraviroc*. The anti-CD4 monoclonal antibody ibalizumab was licensed in 2018, and a variety of additional small molecules that bind to HIV-1 co-receptors are currently in clinical trials.

PRINCIPLES OF THERAPY

The principles of therapy for HIV infection have been articulated by a panel sponsored by the U.S. Department of Health and Human Services as a working group of the NIH Office of AIDS Research Advisory Council. These principles are summarized in **Table 197-23**. As noted in these guidelines, cART of HIV infection does not lead to eradication or cure of HIV. The single possible exception to this is an individual with HIV infection who received an allogeneic stem cell transplant for treatment of acute myelogenous leukemia. His conditioning regimen included cytotoxic chemotherapy, total-body irradiation, and antithymocyte immunoglobulin. The donor cells were homozygous for the CCR5Δ32 mutation (see above) and thus resistant to HIV infection. Despite cART being stopped the day of the transplant, the patient has exhibited no signs of active HIV infection for more than 8 years.

Treatment decisions must take into account the fact that one is dealing with a chronic infection that requires daily therapy. Patients initiating antiretroviral therapy must be willing to commit to lifelong treatment and understand the importance of adherence to their prescribed regimen. The importance of adherence is illustrated by the observation that treatment interruption is associated with rapid increases in HIV RNA levels, rapid declines in CD4+ T cell counts, and an increased risk of clinical progression. While it seems reasonable to assume that the complications associated with cART could be minimized by intermittent treatment regimens designed to minimize exposure to the drugs in question, all efforts to do so have paradoxically been associated with an increase in serious adverse events in the patients randomized to intermittent therapy, suggesting that some "non-AIDS-associated" serious adverse events such as heart attack and stroke may be linked to HIV replication. Thus, unless contraindicated for reasons of toxicity, patients started on cART should remain on cART.

At present, the U.S. Department of Health and Human Services Guidelines panel recommends that everyone with HIV infection be treated with cART and that therapy be initiated a soon as possible after diagnosis. Therapy has been associated with a decrease in disease progression in patients at all stages of HIV infection and leads to a decrease in the risk of transmission of infection. In addition, one may wish to administer a 6-week course of therapy to uninfected individuals immediately following a high-risk exposure to HIV. The combination of tenofovir and emtricitabine is also indicated for pre-exposure prophylaxis in individuals at high risk of HIV

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TABLE 197-22 Combination Formulations of Antiretroviral Drugs

NAME	COMBINATION
Atripla*	Tenofovir disoproxil fumarate + emtricitabine + efavirenz
Biktarvy*	Tenofovir alafenamide + emtricitabine + bictegravir
Cimduo	Tenofovir disoproxil fumarate + lamivudine
Combivir	Zidovudine + lamivudine
Complera*	Tenofovir disoproxil fumarate+ emtricitabine + rilpivirine
Descovy	Tenofovir alafenamide + emtricitabine
Dutrebis	Raltegravir + lamivudine
Epzicom	Abacavir + lamivudine
Evotaz	Atazanavir + cobicistat
Genvoya*	Tenofovir alafenamide + emtricitabine + elvitegravir + cobicistat
Juluca	Dolutegravir + rilpivirine
Kaletra	Lopinavir + ritonavir
Odefsey*	Tenofovir alafenamide +emtricitabine + rilpivirine
Prezcobix	Darunavir + cobicistat
Stribild*	Tenofovir disoproxil fumarate + emtricitabine + elvitegravir + cobicistat
Symfi*	Tenofovir disoproxil fumarate + lamivudine + efavirenz (600 mg)
Symfi Lo*	Tenofovir disoproxil fumarate + lamivudine + efavirenz (400 mg)
Triumeq*	Abacavir + lamivudine + dolutegravir
Truvada	Tenofovir disoproxil fumarate + emtricitabine
Trizivir	Zidovudine + lamivudine + abacavir

*Complete, once-daily, single tablet regimens.

infection. For patients diagnosed with an opportunistic infection and HIV infection at the same time, one may consider a 2- to 4-week delay in the initiation of antiretroviral therapy during which time treatment is focused on the opportunistic infection. This delay may decrease the severity of any subsequent immune reconstitution inflammatory syndrome by lowering the antigenic burden of the opportunistic infection. This is particularly true for patients with TB or cryptococcal infections. For patients with advanced HIV infection (CD4+ <50 cells/μL), however, cART should be initiated as soon as possible.

Once the decision has been made to initiate therapy, the health care provider must decide which drugs to use as the first regimen. The decision regarding choice of drugs not only will affect the immediate response to therapy but also will have implications regarding options for future therapeutic regimens. The initial regimen is usually the most effective insofar as the virus has yet to develop significant resistance. HIV is capable of rapidly developing resistance to any single agent, and therapy must be given as a multidrug combination. Given that patients can be infected with viruses that harbor drug resistance mutations, it is recommended that a viral genotype be done prior to the initiation of therapy to optimize the selection of antiretroviral agents. The combination regimens currently recommended for initial therapy in most treatment-naïve patients are listed in **Table 197-24**. It is currently debated whether treatment-naïve individuals with <50 copies/mL of HIV RNA benefit from cART. While these individuals are at low risk of disease progression in the short term, they do have evidence of persistent immune activation that may have long-term consequences. Following the initiation of therapy one should expect a rapid, at least 1-log (tenfold) reduction in plasma HIV RNA levels within 1–2 months and then a slower decline in plasma HIV RNA levels to <50 copies/mL within 6 months. During this same time there should be a rise in the CD4+ T cell count of 100–150/cells μL that is also particularly brisk during the first month of therapy. Subsequently, one should anticipate a CD4+ T cell count increase of 50–100 cells/year until numbers approach normal. Many clinicians feel that failure to achieve these endpoints is an indication for a change in therapy. Other reasons for a change in therapy include a persistently declining CD4+ T cell count, a consistent increase in HIV RNA levels to >200 copies/mL, clinical deterioration, or drug toxicity (**Table 197-25**). As in the case of initiating therapy, changing therapy may have a lasting impact on future therapeutic options. When changing therapy because of treatment failure (clinical progression or worsening laboratory parameters), it is important to attempt to provide a regimen with at least two new active drugs. This decision can be guided by resistance testing (see below). In the patient in whom a change is made for reasons of drug toxicity, a simple replacement of one drug is reasonable. It should be stressed that in attempting to sort out a drug toxicity it may be advisable to hold all therapy for a period of time to distinguish between drug toxicity and disease progression. Drug toxicity will usually begin to show signs of reversal within 1–2 weeks. Prior to changing a treatment regimen because of drug failure, it is important to ensure that the patient has been adherent to the prescribed regimen. As in the case of initial therapy, the simpler the new therapeutic regimen, the easier it is for the patient to be compliant. Plasma HIV RNA levels should be monitored every 3–6 months during therapy and more frequently if one is contemplating a change in regimen due to an increase in viral load or immediately following a change in regimen.

In order to determine an optimal therapeutic regimen for initial therapy or for a patient on a failing regimen, one may attempt to measure antiretroviral drug susceptibility through genotyping or phenotyping of HIV quasispecies and to determine adequacy of dosing through measurement of drug levels. Genotyping may be done through cDNA sequencing. Phenotypic assays typically measure the enzymatic activity of viral enzymes in the presence or absence of different concentrations of different drugs and have also been used to determine co-receptor tropism. These assays will generally detect quasispecies present at a frequency of ≥10%. Next-generation sequencing may allow detection of quasispecies at frequencies down to 1%. It is generally recommended that resistance testing be used in selecting initial therapy in settings where the risk of transmission of resistant virus is high (such as the United States and Europe) and in determining new regimens for patients experiencing virologic failure while on therapy. Resistance testing may be of particular value in distinguishing drug-resistant virus from poor patient compliance. Due to the rapid rate at which drug-resistant viruses revert to wild-type, it is recommended that resistance testing performed in the setting of drug failure be carried out while the patient is still on the failing regimen. Measurement of plasma drug levels can also be used to tailor an individual treatment. The inhibitory quotient, defined as the trough blood level/IC_{50} of the patient's virus, is used by some to determine the adequacy of dosing of a given treatment regimen. Despite the best of efforts there will still be patients with ongoing high levels of HIV replication while receiving the best available therapy. These patients will receive benefit from remaining on antiretroviral therapy even though it is not fully suppressive.

In addition to the licensed medications discussed above, a large number of experimental agents are being evaluated as possible therapies for HIV infection. Therapeutic strategies are being developed to interfere with virtually every step of the replication cycle of the virus (Fig. 197-3) and in an attempt to eliminate the reservoir of infected cells to "cure" HIV infection. In addition to directly acting antiviral drugs, other strategies, generically referred to as "immune-based therapies," are being developed as a complement to antiviral therapy. Among the antiviral agents in early clinical trials are additional nucleoside and nucleotide analogues, protease inhibitors, fusion inhibitors, receptor and co-receptor antagonists, and integrase inhibitors—as well as new antiviral strategies including antisense nucleic acids and maturation inhibitors. Among the immune-based therapies being evaluated are monoclonal antibodies, IFN-α, bone marrow transplantation, adoptive transfer of lymphocytes genetically modified to resist infection or enhance HIV-specific immunity, active immunotherapy with inactivated HIV or its components, IL-7, and IL-15. Strategies directed toward cure are examining the role of latency-reversing agents such as histone-deacetylase inhibitors.

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HIV AND THE HEALTH CARE WORKER

Health care workers, especially those who deal with large numbers of HIV-infected patients, have a small but definite risk of becoming infected with HIV as a result of professional activities (see "Occupational Transmission of HIV: Health Care Workers, Laboratory Workers, and the Health Care Setting," above).

In the United States 58 health care workers for whom case investigations have been completed have had documented seroconversions to HIV following occupational exposures. Only one of these has occurred since 1999. Approximately 85% of the exposures resulting in infection have been due to percutaneous (puncture/cut injury) exposures to HIV-infected blood. The individuals with documented seroconversions included 19 laboratory workers (16 of whom were clinical laboratory workers), 24 nurses, 16 clinical laboratory technicians, 6 physicians, 4 nonclinical laboratory technicians, 2 housekeepers, 2 surgical technicians, 1 dialysis technician, 1 respiratory therapist, 1 health aide, 1 embalmer/morgue technician, and 1 unknown. In addition, at least 150 possible cases of occupationally acquired HIV infection have been reported among

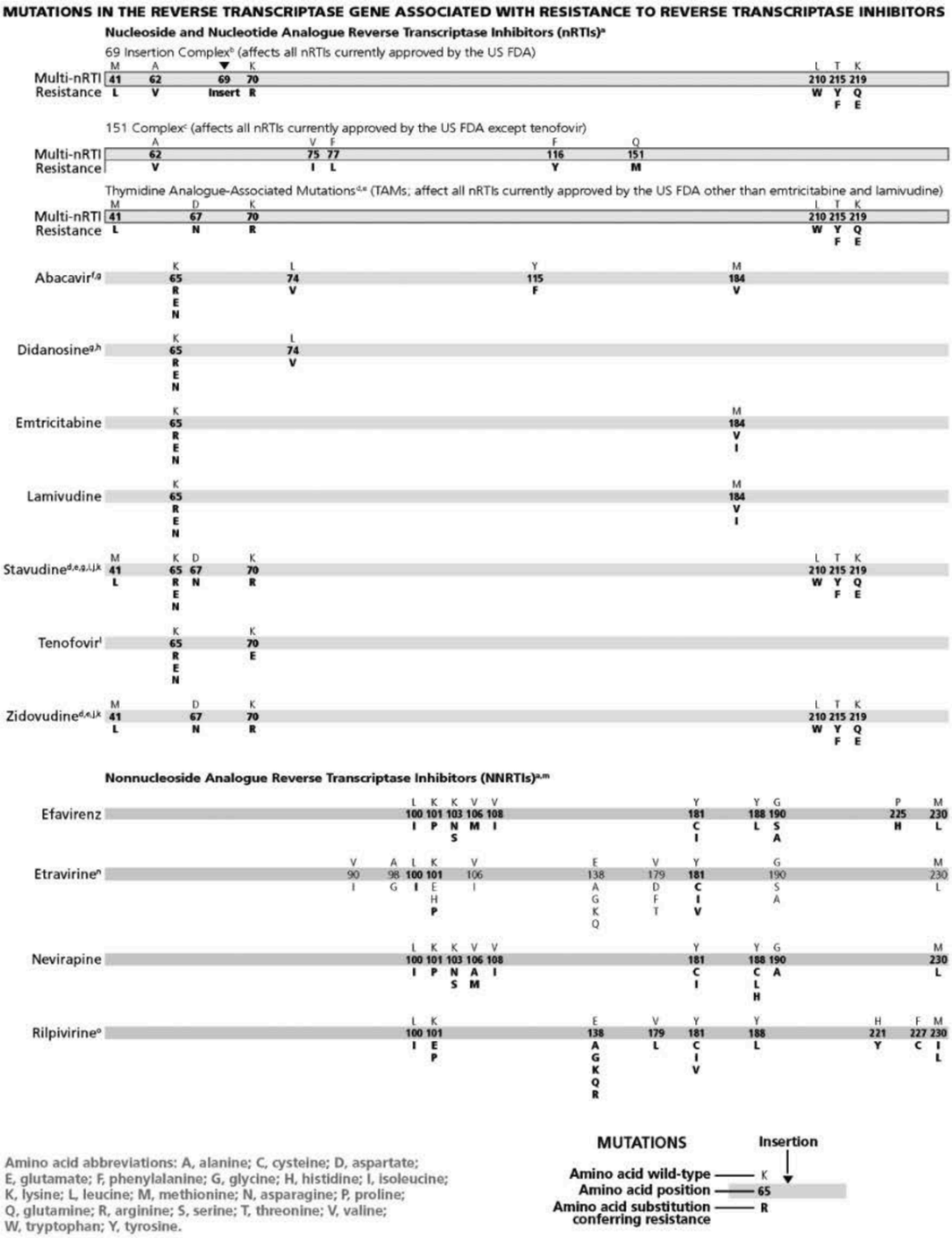

FIGURE 197-47 Amino acid substitutions conferring resistance to antiretroviral drugs. For each amino acid residue, the letter above the bar indicates the amino acid associated with wild-type virus and the letter(s) below indicate the substitution(s) that confer viral resistance. The number shows the position of the mutation in the protein. Mutations selected by protease inhibitors in Gag cleavage sites are not listed. HR1, first heptad repeat; NAMs, nRTI-associated mutations; nRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor. Amino acid abbreviations: A, alanine; C, cysteine; D, aspartate; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine. *(Reprinted with permission from the International Antiviral Society—USA. AM Wensing, V Calvez, HR Günthard et al: 2014 Update of the Drug Resistance Mutations in HIV-1. Top Antivir Med 22:642, 2014. Updated information [and thorough explanatory notes] available at www.iasusa.org.)*

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MUTATIONS IN THE PROTEASE GENE ASSOCIATED WITH RESISTANCE TO PROTEASE INHIBITORS[p,q,r]

Atazanavir +/- ritonavir[s]	L	G	K	L	V	L	E	M	M	G	I	F	I	D	I	I	A	G	V	I	I	N	L	I
	10	16	20	24	32	33	34	36	46	48	**50**	53	54	60	62	64	71	73	82	**84**	85	**88**	90	93
	I	E	R	I	I	I	Q	I	I	V	L	L	L	E	V	L	V	C	A	V	V	S	M	L
	F		M			F		L	L			Y	V			M	I	S	T					M
	V		I			V		V					M			V	T	T	F					
	C		T										T				L	A	I					
Darunavir/ ritonavir[t]	V				V	L			I		I		I					T	V	I			L	
	11				32	33			**47**		**50**		**54**					**74**	**82**	**84**			89	
	I				I	F			V		V		M					P	A	V			V	
													L						T					
Fosamprenavir/ ritonavir	L				V				M	I	I		I					G	V	I			L	
	10				32				46	47	**50**		54					73	82	**84**			90	
	F				I				I	V	V		L					S	A	V			M	
	I								L				V						F					
	R												M						S					
	V																		T					
Indinavir/ ritonavir[u]	L		K	L	V		M	M					I				A	G	V	I			L	
	10		20	24	32		36	**46**					54				71	73	**82**	**84**			90	
	I		M	I	I		I	I					V				V	S	A	V			M	
	R		R					L									T	A	F					
	V																		T					
Lopinavir/ ritonavir[v]	L		K	L	V	L		M	I		I	F	I		L		A	G	L	V	V	I		L
	10		20	24	**32**	33		46	**47**		50	53	54		63		71	73	**76**	**77**	**82**	84		90
	F		M	I	I	F		I	V		V	L	V		P		V	S	V	I	A	V		M
	I		R					L	A				L				T				F			
	R												A								T			
	V												M								S			
													T											
													S											
Nelfinavir[u,w]	L				D		M	M									A			V	V	I	N	L
	10				**30**		36	46									71			77	82	84	88	**90**
	F				N		I	I									V			I	A	V	D	M
	I							L									T				F		S	
																					T			
																					S			
Saquinavir/ ritonavir[u]	L			L						G			I		I		A	G		V	V	I		L
	10			24						**48**			54		62		71	73		77	82	84		**90**
	I			I						V			V		V		V	S		I	A	V		M
	R												L				T				F			
	V																				T			
																					S			
Tipranavir/ ritonavir	L					L	M		K	M	I		I	Q		H		T	V	N	I		L	
	10					33	36		43	46	**47**		54	**58**		69		**74**	**82**	83	**84**		89	
	V					F	I		T	L	V		A	E		K		P	L	D	V		I	
							L						M			R			T				M	
							V						V										V	

MUTATIONS IN THE ENVELOPE GENE ASSOCIATED WITH RESISTANCE TO ENTRY INHIBITORS

Enfuvirtide[x]	G	I	V	Q	Q	N	N
	36	**37**	**38**	**39**	**40**	**42**	**43**
	D	**V**	**A**	**R**	**H**	**T**	**D**
	S		**M**				
			E				
Maraviroc[y]	See User Note						

MUTATIONS IN THE INTEGRASE GENE ASSOCIATED WITH RESISTANCE TO INTEGRASE STRAND TRANSFER INHIBITORS[z]

Dolutegravir[aa]	F	E	G	Q	N	R
	121	138	140	**148**	155	263
	Y	A	A	**H**	H	K
		K	S	**K**		
				R		
Elvitegravir[bb]	T	E	T	F	S	Q	N	R
	66	**92**	97	**121**	**147**	**148**	**155**	263
	I	**Q**	A	**Y**	**G**	**H**	**H**	K
	A	G				**K**		
	K					**R**		
Raltegravir[cc]	L	E	T	F	E	G	Y	Q	N	R
	74	92	97	**121**	138	140	**143**	**148**	**155**	263
	M	Q	A	**Y**	A	A	**R**	**H**	**H**	K
					K	S	**H**	**K**		
							C	**R**		

FIGURE 197-47 (*Continued*)

health care personnel in the United States. The number of these workers who actually acquired their infection through occupational exposures is not known. Taken together, data from several large studies suggest that the risk of HIV infection following a percutaneous exposure to HIV-contaminated blood is ~0.2323%, and after a mucous membrane exposure, ~0.09%. Although episodes of HIV transmission after nonintact skin exposure have been documented, the average risk for transmission by this route has not been precisely quantified but is estimated to be less than the risk for mucous membrane exposures. The risk for transmission after exposure to body fluids or tissues other than HIV-infected blood also has not been quantified but is probably considerably lower than for blood exposures. A seroprevalence survey of 3420 orthopedic surgeons, 75% of whom practiced in an area with a relatively high prevalence of HIV infection and 39% of whom reported percutaneous exposure to patient blood, usually through an accident involving a suture needle, failed to reveal any cases of possible occupational infection, suggesting that the risk of infection with a suture needle may be considerably less than that with a blood-drawing (hollow-bore) needle.

Most cases of health care worker seroconversion occur as a result of needle-stick injuries. When one considers the circumstances that result in needle-stick injuries, it is immediately obvious that adhering to the standard guidelines for dealing with sharp objects would result in a significant decrease in this type of accident. In one study, 27% of needle-stick injuries resulted from improper disposal of the needle (over half of these were due to recapping the needle), 23% occurred during attempts to start an IV line, 22% occurred during blood drawing, 16% were associated with an IM or SC injection, and 12% were associated with giving an IV infusion.

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TABLE 197-23 Principles of Therapy of HIV Infection

1. Ongoing HIV replication leads to immune system damage, progression to AIDS, and systemic immune activation.
2. Plasma HIV RNA levels indicate the magnitude of HIV replication and the rate of CD4+ T cell destruction. CD4+ T cell counts indicate the current level of competence of the immune system.
3. Maximal suppression of viral replication is a goal of therapy; the greater the suppression the less likely the appearance of drug-resistant quasispecies.
4. The most effective therapeutic strategies involve the simultaneous initiation of combinations of effective anti-HIV drugs with which the patient has not been previously treated and that are not cross-resistant with antiretroviral agents that the patient has already received.
5. The antiretroviral drugs used in combination regimens should be used according to optimum schedules and dosages.
6. The number of available drugs is limited. Any decisions on antiretroviral therapy have a long-term impact on future options for the patient.
7. Women should receive optimal antiretroviral therapy regardless of pregnancy status.
8. The same principles apply to children and adults. The treatment of HIV-infected children involves unique pharmacologic, virologic, and immunologic considerations.
9. Compliance is an important part of ensuring maximal effect from a given regimen. The simpler the regimen, the easier it is for the patient to be compliant.

Source: Modified from *Principles of Therapy of HIV Infection*, USPHS, and the Henry J. Kaiser Family Foundation.

Occupational exposures to HIV should be considered as a medical emergency to ensure timely postexposure management and administration of postexposure antiretroviral prophylaxis (PEP). Recommendations regarding PEP must take into account that a variety of circumstances determine the risk of transmission of HIV following occupational exposure. In this regard, several factors have been associated with an increased risk for occupational transmission of HIV infection, including deep injury, the presence of visible blood on the instrument causing the exposure, injury with a device that had been placed in the vein or artery of the source patient, and advanced HIV disease in the source patient. Other important considerations when considering PEP in the health care worker include known or suspected pregnancy or breast-feeding, the possibility of exposure to drug-resistant virus, and the toxicities of different PEP regimens. Regardless of the decision to use PEP, the wound should be cleansed immediately and antiseptic applied. If a decision is made to offer PEP, U.S. Public Health Service guidelines recommend that PEP regimens contain 3 (or more) antiretroviral drugs administered for a 4-week duration for all occupational exposures to HIV. Detailed guidelines are available from the *Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HIV and Recommendations for Postexposure Prophylaxis* (CDC, 2013). The report emphasizes the importance of adherence to PEP when it is indicated, and close follow-up of exposed workers should be provided including counseling, baseline and follow-up HIV testing, and monitoring for drug toxicity. Follow-up appointments should begin within 72 h of an HIV exposure, and if newer fourth-generation combination HIV p24 antigen-HIV antibody test is utilized for follow-up HIV testing of the exposed health care worker, HIV testing may be concluded 4 months after exposure; if a newer testing platform is not available, follow-up HIV testing is typically concluded 6 months after an HIV exposure. For consultation on the treatment of occupational exposures to HIV and other bloodborne pathogens, the clinician managing the exposed patient can call the National Clinicians' Post-Exposure Prophylaxis Hotline (PEPline) at 888-448-4911. This service is available 24 hours a day at no charge. (Additional information on the Internet is available at *www.nccc.ucsf.edu*.) PEPline support may be especially useful in challenging situations, such as when drug-resistant HIV strains are suspected or the health care worker is pregnant.

TABLE 197-24 Initial Combination Regimens Recommended for Most Treatment-Naïve Patients Regardless of HIV RNA Level or CD4 Count

Dolutegravir + tenofovir* + emtricitabine**
Raltegravir + tenofovir* + emtricitabine**
Elvitegravir + tenofovir*+ emtricitabine**
Elvitegravir + cobicistat + tenofovir* + emtricitabine**
Dolutegravir + abacavir + lamivudine** (only for those HLA-B*5701 negative)

*Tenofovir alafenamide and tenofovir disoproxil fumarate are two forms of tenofovir approved by FDA. Tenofovir alafenamide has fewer bone and renal toxicities while tenofovir disoproxil fumarate is associated with lower lipid levels. **Lamivudine may substitute for emtricitabine and vice versa.

Source: Guidelines for the Use of Antiretroviral Agents in HIV-Infected Adults and Adolescents, USPHS.

TABLE 197-25 Indications for Changing Antiretroviral Therapy in Patients with HIV Infection[a]

Less than a 1-log drop in plasma HIV RNA by 4 weeks following the initiation of therapy
A reproducible significant increase (defined as threefold or greater) from the nadir of plasma HIV RNA level not attributable to intercurrent infection, vaccination, or test methodology
Persistently declining CD4+ T cell numbers
Clinical deterioration
Side effects

[a]Generally speaking, a change should involve the initiation of at least two drugs felt to be effective in the given patient. The exception to this is when change is being made to manage toxicity, in which case a single substitution is reasonable.

Source: Guidelines for the Use of Antiretroviral Agents in HIV-Infected Adults and Adolescents, USPHS.

Health care workers can minimize their risk of occupational HIV infection by following the CDC guidelines of June 2015, which include adherence to universal precautions, assuming that blood and other body fluids from all patients are potentially infectious. Therefore, the following infection control precautions should be adhered to at all times: (1) routinely use barriers (such as gloves and/or goggles) when anticipating contact with blood or body fluids; (2) immediately wash hands and other skin surfaces after contact with blood or body fluids; and (3) carefully handle and dispose of sharp instruments during and after use. For further information contact the CDC at 800-CDC-INFO (232-4636) or see *www.cdc.gov/info*. In attempting to put this small but definite occupational risk of HIV infection to the health care worker in perspective, it is important to point out that ~200 health care workers die each year as a result of occupationally acquired hepatitis B infection. The tragedy in this instance is that these infections and deaths due to HBV could be greatly decreased by more extended use of the HBV vaccine. The risk of HBV infection following a needle-stick injury from a hepatitis antigen–positive patient is much higher than the risk of HIV infection (see "Transmission," above). There are multiple examples of needle-stick injuries where the patient was positive for both HBV and HIV and the health care worker became infected only with HBV. For these reasons, it is advisable, given the high prevalence of HBV infection in HIV-infected individuals, that all health care workers dealing with HIV-infected patients be immunized with the HBV vaccine.

TB is another infection common to HIV-infected patients that can be transmitted to the health care worker. For this reason, all health care workers should know their PPD status, have it checked yearly, and, where appropriate, receive 6 months of isoniazid treatment if their skin test converts to positive. In addition, all patients in whom a diagnosis of pulmonary TB is being entertained should be placed immediately in respiratory isolation, pending results of the diagnostic evaluation. The emergence of drug-resistant organisms, including extensively drug-resistant TB strains, has made TB an increasingly important problem for health care workers. This is particularly true for the health care worker with preexisting HIV infection.

One of the most charged issues ever to come between health care workers and patients is that of transmission of infection from HIV-infected health care workers to their patients. This is discussed in "Occupational Transmission of HIV: Health Care Workers, Laboratory Workers, and the Health Care Setting," above. Theoretically, the same universal precautions that are used to protect the health care worker

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 from the HIV-infected patient will also protect the patient from the HIV-infected health care worker.

VACCINE FOR THE PREVENTION OF HIV INFECTION

There is currently no safe and effective vaccine that has been approved for the prevention of HIV infection. Successful vaccines are predicated on the assumptions that the body can mount an adequate immune response to the microbe or virus in question during natural infection and that the vaccine will mimic the natural response to infection. Even with serious diseases, such as smallpox, poliomyelitis, measles, and influenza among others, the body in the vast majority of cases clears the infectious agent and provides protection, which is usually life-long against future exposure against the same pathogen. Unfortunately, this is not the case with HIV infection since the natural immune response to HIV infection is unable to clear the virus from the body and cases of superinfection are not uncommon. Some of the factors that contribute to the problematic nature of development of a preventive HIV vaccine are (1) the high mutability of the virus; (2) the fact that the infection can be transmitted by cell-free or cell-associated virus; (3) the fact that the HIV provirus integrates itself into the genome of the target cell and may remain in a latent form unexposed to the immune system; (4) the likely need for the development of effective mucosal immunity; and (5) the fact that it has been difficult to establish the precise correlates of protective immunity to HIV infection. A fraction of a percent of HIV-infected individuals are "elite controllers" in that they maintain extremely low and even undetectable levels of viremia in the absence of cART, and a number of individuals have been exposed to HIV multiple times but remain uninfected; these facts suggest that there are elements of host defense or an HIV-specific immune response that have the potential to be protective against acquisition of infection. Early attempts to develop a vaccine with the envelope protein gp120 aimed at inducing neutralizing antibodies in humans were unsuccessful; the elicited antisera failed to neutralize primary isolates of HIV cultured and tested in fresh peripheral blood mononuclear cells. In this regard, two phase 3 trials were undertaken in the United States and Thailand using soluble gp120, and the vaccines failed to protect human volunteers from HIV infection. In addition, two separate vaccine trials aimed at eliciting CD8+ T cell responses to prevent infection and, if unsuccessful in preventing infection, to control postinfection viremia, also failed at both goals. In 2009, a vaccine using a poxvirus vector prime expressing various viral proteins followed by an envelope protein boost was tested in a 16,000-person clinical trial (RV144) conducted in Thailand among predominantly low-HIV-prevalence heterosexuals. The vaccine provided the first positive, albeit very modest, signal ever reported in an HIV vaccine trial, showing 31% protection against acquisition of infection. Such a result is certainly not sufficient justification for clinical use of the vaccine, but it served as an important first step in the direction of the development of a safe and effective vaccine against HIV infection. Follow-up studies of RV144 indicate that non-neutralizing or weakly neutralizing antibody responses against certain constant epitopes in the otherwise highly variable V1-V2 region of the HIV envelope may be associated with the modest degree of protection observed in that clinical trial. Additional similar studies are being conducted in high-HIV-prevalence countries in sub-Saharan Africa in attempts to improve on the results of RV144 by a variety of approaches, including increasing the number of vaccine boosts with envelope protein and the addition of adjuvant.

An area of HIV vaccine research that is currently being actively pursued is the attempt to induce broadly neutralizing antibodies by developing as immunogens for vaccination certain epitopes on the HIV envelope that are the targets of naturally occurring broadly neutralizing antibodies during HIV infection. It is curious that only about 20% of HIV-infected individuals develop broadly neutralizing antibodies in response to natural infection and they do so only after 2–3 years of ongoing infection. By the time these antibodies appear, they can neutralize a broad range of primary HIV isolates, but they appear to be ineffective against the autologous virus in the infected subject. Upon close examination, these broadly neutralizing antibodies manifest a high degree of somatic mutations that were accumulated over time and are responsible for their affinity maturation and broadly neutralizing capacity. The goal of current efforts is to develop the conformationally correct HIV envelope epitopes that, when used as immunogens, would direct the immune response of an uninfected individual to the production of broadly neutralizing antibodies over a reasonable time frame by sequential immunizations. It remains to be seen whether this approach will be feasible.

PREVENTION OF HIV ACQUISITION

Education, counseling, and behavior modification are the cornerstones of any HIV prevention strategy. A major problem in the United States and elsewhere is that many infections are passed on by those who do not know that they are infected. Of the ~1.1 million persons in the United States who are HIV-infected, it is estimated that ~15% do not know their HIV status and approximately 23% of all new infections are transmitted by those people who are not aware that they are infected. In this regard, the CDC has recommended that HIV testing become part of routine medical care and that all individuals between the ages of 13 and 64 years be tested at least one time. These individuals should be informed of the testing and be tested without the need for written informed consent. Each individual can "opt out" of testing, but testing should otherwise be routinely administered. Individuals who are practicing high-risk behavior should be tested more often. In addition to identifying individuals who might benefit from cART, information gathered from such an approach should serve as the basis for behavior-modification programs, both for infected individuals who may be unaware of their HIV status and who could infect others and for uninfected individuals practicing high-risk behavior. The practice of "safer sex" is the most effective way for sexually active uninfected individuals to avoid contracting HIV infection and for infected individuals to avoid spreading infection. Abstinence from sexual relations is the only absolute way to prevent sexual transmission of HIV infection. However, for most individuals this is not feasible, and there are a number of relatively safe practices that can markedly decrease the chances of transmission of HIV infection. Partners engaged in monogamous sexual relationships who wish to be assured of safety should both be tested for HIV antibody. If both are negative, it must be understood that any divergence from monogamy puts both partners at risk; open discussion of the importance of honesty in such relationships should be encouraged. When the HIV status of either partner is not known, or when one partner is positive, there are a number of options. Use of condoms can markedly decrease the chance of HIV transmission. It should be remembered that condoms are not 100% effective in preventing transmission of HIV infection, and there is a ~10% failure rate of condoms used for contraceptive purposes. Most condom failures result from breakage or improper usage, such as not wearing the condom for the entire period of intercourse. Latex condoms are preferable, since virus has been shown to leak through natural skin condoms. Petroleum-based gels should never be used for lubrication of the condom, since they increase the likelihood of condom rupture. Some men who have sex with men practice exclusively fellatio as a "minimal-risk" activity compared with anal intercourse. It should be emphasized that receptive fellatio is not guaranteed safe sex, and although the incidence of transmission via fellatio is considerably less than that of rectal or vaginal intercourse, there has been documentation of transmission of HIV where receptive fellatio was the only sexual act performed (see "Transmission," above). Topical microbicides composed of gels containing antiretroviral drugs have been shown to be only modestly and variably efficacious in preventing acquisition of HIV infection in women engaging in vaginal intercourse. The considerable degree of variability in efficacy relates to the generally poor adherence of participants to the use of the intervention. Pre-exposure prophylaxis (PrEP) using oral antiretroviral drugs such as Truvada (tenofovir + emtricitabine) as a single daily pill in uninfected men who have sex with men and transgender women is highly efficacious in preventing acquisition of HIV infection. The degree of efficacy can be greater than 95% if subjects adhere strictly to the regimen. CDC estimates that approximately 1.2 million people in the United States are at "substantial risk" for HIV infection and should be counseled about PrEP. As of 2016, however, fewer than half of primary physicians and nurses have heard of PrEP.

Adult male circumcision, which has been shown to result in a 50–65% reduction in HIV acquisition in the circumcised subject, is

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currently being pursued, particularly in developing nations, as a component of HIV prevention. The most effective way to prevent transmission of HIV infection among IDUs is to stop the use of injectable drugs. Unfortunately, that is extremely difficult to accomplish unless the individual enters a treatment program. For those who will not or cannot participate in a drug treatment program and who will continue to inject drugs, the avoidance of sharing of needles and other paraphernalia ("works") is the next best way to avoid transmission of infection. However, the cultural and social factors that contribute to the sharing of paraphernalia are complex and difficult to overcome. Under these circumstances, paraphernalia should be cleaned after each usage with a virucidal solution, such as undiluted sodium hypochlorite (household bleach). Needle exchange programs have been highly successful in decreasing HIV transmission among injection drug users without increasing the use of injection drugs. Oral PreP also is effective in preventing acquisition of HIV infections among IDUs. It is important for IDUs to be tested for HIV infection and counseled to avoid transmission to their sexual partners. Prevention of transmission through blood or blood products and prevention of mother-to-child transmission are discussed in "Transmission," above.

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Section 15 **Infections Due to RNA Viruses**

198 Viral Gastroenteritis

Umesh D. Parashar, Roger I. Glass

Acute infectious gastroenteritis is a common illness that affects persons of all ages worldwide. It is a leading cause of death among children in developing countries, accounting for an estimated 0.6 million deaths each year, and is responsible for up to 10–12% of all hospitalizations among children in industrialized countries, including the United States. Elderly persons, especially those with debilitating health conditions, also are at risk of severe complications and death from acute gastroenteritis. Among healthy young adults, acute gastroenteritis is rarely fatal but incurs substantial medical and social costs, including those of time lost from work.

Several enteric viruses have been recognized as important etiologic agents of acute infectious gastroenteritis (**Table 198-1**, **Fig. 198-1**). Although most viral gastroenteritis is caused by RNA viruses, the DNA viruses that are occasionally involved (e.g., adenovirus types 40 and 41) are included in this chapter. Illness caused by these viruses is characterized by the acute onset of vomiting and/or diarrhea, which may be accompanied by fever, nausea, abdominal cramps, anorexia, and malaise. As shown in **Table 198-2**, several features can help distinguish gastroenteritis caused by viruses from that caused by bacterial agents. However, the distinction based on clinical and epidemiologic parameters alone is often difficult, and laboratory tests are required to confirm the diagnosis.

HUMAN CALICIVIRUSES

Etiologic Agent The Norwalk virus is the prototype strain of a group of small (27–40 nm), nonenveloped, round, icosahedral viruses with relatively amorphous surface features on visualization by electron microscopy. Molecular cloning and characterization have demonstrated that the viruses have a single, positive-strand RNA genome ~7.5 kb in length and possess a single virion-associated protein—similar to that of typical caliciviruses—with a molecular mass of 60 kDa. On the basis of these molecular characteristics, these viruses are presently classified into two genera belonging to the family Caliciviridae: the *noroviruses* and the *sapoviruses* (previously called Norwalk-like viruses and Sapporo-like viruses, respectively). Human noroviruses can be classified into three genogroups: GI, GII, and GIV, which include 9, 22, and 1 genotype, respectively.

Epidemiology Infections with the Norwalk and related human caliciviruses are common worldwide, and most adults have antibodies to these viruses. Antibody is acquired at an earlier age in developing countries—a pattern consistent with the presumed fecal–oral mode of transmission. Infections occur year-round, although, in temperate climates, a distinct increase has been noted in cold-weather months. Noroviruses may be the most common infectious agents of mild gastroenteritis in the community and affect all age groups, whereas sapoviruses primarily cause gastroenteritis in children. Noroviruses also cause traveler's diarrhea, and outbreaks have occurred among military personnel deployed to various parts of the world. The limited data available indicate that norovirus may be the second most common viral agent (after rotavirus) among young children and the most common agent among older children and adults. In the United States, with the decline in severe rotavirus disease following implementation of a rotavirus vaccination program, norovirus has become the leading cause of medically attended gastroenteritis in young children. Noroviruses are also recognized as the major cause of epidemics of gastroenteritis worldwide. In the United States, ~50% of all reported outbreaks of gastroenteritis are caused by noroviruses.

Virus is transmitted predominantly by the fecal–oral route but is also present in vomitus. Because an inoculum with very few viruses can be infectious, transmission can occur by aerosolization, by contact with contaminated fomites, and by person-to-person contact. Viral shedding and infectivity are greatest during the acute illness, but challenge studies with Norwalk virus in volunteers indicate that viral antigen may be shed by asymptomatically infected persons and also by symptomatic persons before the onset of symptoms and for several weeks after the resolution of illness. Viral shedding can be prolonged in immunocompromised individuals.

Pathogenesis The exact sites and cellular receptors for attachment of viral particles have not been determined. Data suggest that carbohydrates that are similar to human histo-blood group antigens and are present on the gastroduodenal epithelium of individuals with the secretor phenotype may serve as ligands for the attachment of Norwalk virus. Additional studies must more fully elucidate norovirus–carbohydrate interactions, including potential strain-specific variations. After

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Mon, 7 May 2018 01:48:56 +0000
To: Chun, Tae-Wook (NIH/NIAID) [E]
Subject: FW: New Paper_Stress and AMPK links HIV, Jumping Genes, & Learning/Memory
Attachments: Detailed write-up_References.doc, Detailed write-up.doc, Final Paper_LTP-HIV_AMPK_Print version.pdf

Who is this?

From: Jahahreeh Finley (b)(6)
Sent: Saturday, May 05, 2018 10:07 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: New Paper_Stress and AMPK links HIV, Jumping Genes, & Learning/Memory

Dear Dr. Fauci,

My name is Jahahreeh Finley and I am a graduate of Johns Hopkins University and I've had several email exchanges with Tae-Wook Chun in the past about the potential of AMPK activators including metformin to reactivate latent HIV-1, possibly contributing to a cure.

As T cell activation is associated with a transient increase in telomerase, I wanted to forward over some information from a recent study and my new paper that was recently published showing for the first time that metformin activates human telomerase (hTERT) in human aortic endothelial cells (HAECs) and significantly delays endothelial senescence in an AMPK-dependent manner [1,2]. Although the study that showed this was first published in January of 2018, in my new paper I actually first proposed that metformin would activate telomerase via AMPK activation (my paper was under review for almost a year). Interestingly, transfection of hTERT was shown to reverse senescence in Hutchinson-Gilford progeria syndrome (HGPS) cells and metformin alleviates accelerated aging defects and activates AMPK in HGPS cells [4-6].

My new paper links two Nobel Prize-winning discoveries with the creation of human life, the creation of new species, how we learn & create new memories, and the potential eradication of HIV via AMPK activation. The way I came up with the idea that AMPK activation by cellular stress-inducing compounds like metformin would activate telomerase is that telomerase itself is derived from a transposable element, also known as a "jumping gene", which was first noted in the landmark initial sequencing of the human genome in 2001 by the NIH [3].

I don't quite remember how, but I stumbled across the Noble Prize speech given by Barbara McClintock in 1983 for her discovery of "jumping genes" in the 1950s. Jumping genes make up almost half of the human genome and can "jump" from one spot to another on a chromosome. These jumping genes can jump right next to or right in the middle of another gene. Such gene jumping can actually create new genes and new species that are stronger and more resilient than their parents.

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In her Nobel Prize speech, McClintock said that it seemed as if a "challenge" or a "shock" to jumping genes is what caused them to jump to new spots and create new species. I had no idea that I was using almost the same exact words, that this Nobel Prize winner was using, to describe what creates all human life and is responsible for learning and the creation of memories, what I first called the "shock and live" approach. This same "shock" that McClintock referred to is also what HIV-1 researchers are using to try and cure HIV-1, called the "shock and kill" approach.

My paper is the first to make the connection that the same compounds/stressors that McClintock talked about are the same stressors that induce a "shock" to both human oocytes and human sperm, creating normal healthy babies and are used by HIV cure researchers to "shock" HIV back to life to be killed. These same compounds/stressors also "shock" neurons in an area of the brain called the hippocampus, where a good portion of learning and memory take place. These same compounds/stressors also "shock" jumping genes, causing them to jump from one spot to another, causing many organisms to become stronger, more resilient, and even create new species.

Even more astonishing is that jumping genes have been found in the hippocampus of the human brain, in human sperm, and in human oocytes. My paper is also the first to make the connection that activation of AMPK by many stress-inducing compounds, including metformin and many plant-derived compounds, is what helps "shock" neurons in the brain to help us learn, "shocks" human oocytes to help them "wake up" (similar to dormant HIV), and "shocks" human sperm to help them release enzymes that are necessary to penetrate and fertilize an egg. Both AMPK and jumping genes have been shown to help oocytes become fully mature in preparation for fertilization. Exercise and an intellectually stimulating environment also cause jumping genes to jump in the brain, helping to form memories. Metformin and AMPK also inhibit Zika, Dengue, malaria, and *Legionella pneumophila*.

Lastly, I also proposed that this "shock" probably governs everything we consider to be alive. For example, about 70% of the antibiotics that we use in the clinic come from one bacterial genus (*Streptomyces*). The way to get these bacteria to produce more antibiotics is to "shock" them (e.g. starvation, heat stress, etc.). Basically, these bacteria are at their best (i.e. producing more antibiotics) when they are challenged. Interestingly, an antibiotic produced by these bacteria, called ionomycin, activates jumping genes, helps "wake up" dormant HIV, activates AMPK in neurons, "shocks" human sperm to release egg-penetrating enzymes, and is used in fertility clinics all over the world to activate human oocytes, producing normal, healthy children.

I've attached a slightly more detailed write-up that includes all of the preceding information with the associated references in addition to my recent publication. I've also included some information that didn't make it into my paper on how AMPK may help sustain suspended animation and biostasis. NASA is very much interested in inducing biostasis to slow biological aging to facilitate transport of animals (and potentially humans) into deep space. DARPA

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(Defense Advanced Research Projects Agency) is also interested in biostasis induction to slow biological time in wounded soldiers on the battlefield to allow more time for transport to a medical facility.

Please let me know at your earliest convenience your thoughts on these novel connections and any suggestions that you may have that could increase exposure and visibility of my work in the science community. Thanks and I hope all is well.

Best,

Jahahreeh Finley
Finley BioSciences
(b)(6)

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I wanted to forward over some information first elucidated in several of my recent publications that highlights a novel link between biostasis/suspended animation and potential virus eradication (i.e. HIV-1), hippocampal long-term potentiation (essential for learning and memory), alleviation of accelerated cellular aging in Hutchinson-Gilford progeria syndrome, oocyte activation and the sperm acrosome reaction (prerequisites for human life creation), and transposable element (i.e. "jumping genes")-mediated promotion of learning, memory, and the creation of human life [1-7].

The link between such disparate physiological and pathophysiological phenomena is cellular stress-induced modulation of energy metabolism, leading to the activation of the master metabolic regulator AMPK, a kinase that increases lifespan and healthspan in several model organisms [24]. I was the first to propose and publish (2014) that an increase in beneficial levels of cellular stress (e.g. increases in the levels reactive oxygen species [ROS], calcium [Ca2+], and/or an AMP(ADP)/ATP ratio increase, etc.) and activation of AMPK by compounds including metformin would alleviate accelerated cellular aging defects in children diagnosed with the genetic disorder Hutchinson-Gilford progeria syndrome (HGPS), a disease characterized by an accelerated aging phenotype and death at ~14.6 years of age [1]. This hypothesis was substantiated in 2016 and 2017, with metformin activating AMPK in cells taken from HGPS kids and ameliorating accelerated cellular aging defects (e.g. correcting nuclear morphology, decrease in senescence markers, etc.) [8,9].

As normal humans make the same toxic protein (called progerin) that leads to accelerated aging in HGPS kids (just at lower amounts that increase with age), AMPK activation links the concept of biostasis induction with decelerated human aging [10]. Indeed, metformin was recently shown to activate telomerase (enzyme that lengthens telomeres) in human cells in an AMPK-dependent manner [11]. AMPK is activated in the freeze-tolerant wood frog in response to anoxia, dehydration, and freezing, indicating that AMPK plays a beneficial role in modulating energy metabolism to enhance survival in response to environmental stressors [12]. AMPK has also been conserved in tardigrades, organisms that exhibit tolerance to the most extreme of environmental stressors by undergoing cryptobiosis and trehalose, a sugar that confers desiccation tolerance in a number of organisms, activates AMPK in human cells and *in vivo* in mice [13,14,41]. AMPK is also activated in the desert snail during profound metabolic rate depression (estivation) and in freeze-tolerant insects that undergo diapause (a period of suspended development) [15,16]. Moreover, *C. elegans* exposed to anoxia enter into a state of suspended animation and metformin increases survival of *C. elegans* exposed to long-term anoxia in an AMPK-dependent manner [17]. Additionally, AMPK is activated during winter hibernation in 13-lined ground squirrels and in the heart of the gray mouse lemur during torpor (decreased physiological state), suggesting that diapause, torpor, and some forms of cryptobiosis are linked by AMPK-regulated metabolic rate depression and extended survival [18,19]. Researchers at NASA are also exploring the concept of inducing "synthetic torpor" to facilitate transportation of experimental animals (and possibly humans) into deep space via metabolic rate depression [20]. Several compounds that were noted in that article as being torpor induction triggers, including 5′-AMP, 3-iodothyronamine, and hydrogen sulfide (H2S) each activate AMPK [20-23].

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Furthermore, AICAR, a widely-used AMPK activator, depresses metabolism and increases freezing survival in human cells and both AICAR and metformin improves *ex-vivo* preservation of rat livers via AMPK when added to tissue preservation solution during cold ischemia [25-27]. Thus, AMPK likely represents a central node that beneficially modulates metabolic rate depression to enhance or extend survival during torpor, cryptobiosis, and hibernation-like states as well as in normal and pathological aging in human cells.

AMPK also links potential virus eradication with biostasis and suspended animation. I first proposed in 2015 that AMPK activation links alleviation of accelerated aging in HGPS with the potential eradication of HIV-1 via the "shock and kill" approach, a method currently being pursued by HIV cure researchers to possibly eradicate HIV-1 [2,28]. The same gene splicing factor that promotes accelerated aging in HGPS (called SRSF1 or ASF/SF2) also inhibits reactivation of latent HIV-1 (i.e. "shock"), preventing immune system detection and virus destruction (i.e. "kill") [8,29]. Metformin was shown to slow aging in HGPS cells by decreasing this splicing factor, as I originally predicted in 2014, and several compounds that potently induce latent HIV-1 reactivation in T cells from infected patients, including PMA (a phorbol ester) combined with ionomycin, each activate AMPK [1,8,30-32]. AMPKα1 deletion leads to a decrease in primary T cell responses to bacterial and viral infections *in vivo*, AMPK knockdown leads to cell death on T cell activation, and metformin has recently been shown to inhibit Zika and Dengue viruses, the malaria parasite, and *Legionella pneumophila* [33-38]. Intriguingly, early data presented at the International AIDS Conference in 2017 demonstrated that metformin destabilized the latent HIV-1 reservoir in chronically-infected HIV patients and decreased the percentage of CD4+ T cells expressing the immune checkpoint receptors PD-1, TIGIT, and TIM-3, each markers associated with T cells latently infected with HIV-1, indicating that AMPK activation may indeed contribute to a cure for HIV-1 [39,40].

The induction of cellular stress and AMPK activation also links biostasis, decelerated aging, and potential HIV-1 eradication with oocyte activation and the sperm acrosome reaction, prerequisites for the creation of human life. Increases in both ROS and Ca2+ are critical for T cell activation (and hence latent HIV-1 reactivation) and ROS is transiently increased in HGPS cells when treated with a rapamycin analog to alleviate accelerated aging [42-44]. Stress-induced activation of AMPK by AICAR and other compounds promotes oocyte maturation, which precedes and is essential for efficient oocyte activation [45,46]. Oocyte activation is indispensable for the creation of all human life and PMA and ionomycin, which collectively reactivates latent HIV-1, activates mouse and human oocytes, respectively [47,48]. AMPK is also found in the acrosome of the human sperm head and ionomycin induces the acrosome reaction in human sperm, a process necessary for oocyte penetration and fertilization [49,50]. Ionomycin is also used extensively during fertility procedures to activate human oocytes (i.e. "shock"), creating normal, healthy children (i.e. "live") [48]. Interestingly, ionomycin is a narrow spectrum antibiotic produced by certain species within the bacterial genus *Streptomyces*, from which ~70 percent of clinically useful antibiotics are derived [51,52]. Cellular stress, mediated by increases in ROS, Ca2+, and/or an

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AMP(ADP)/ATP ratio increase, etc. also enhances antibiotic production in many *Streptomyces* strains, reinforcing the notion that the beneficial effects of cellular stress induction crosses species boundaries [53,54].

Cellular stress induction and AMPK activation also play a critical role in learning and memory. Hippocampal long-term potentiation (LTP) is considered the cellular correlate of learning and memory and AMPK has been found localized in hippocampal CA1 dendrites and is activated in neurons by metformin, AICAR, ionomycin, and glutamate, a neurotransmitter essential for hippocampal LTP induction [55-57]. The glutamate receptors AMPAR and NMDAR are found on and modulate T cell activation, AMPK activation increases synthesis and membrane insertion of AMPARs (critical for LTP expression), PMA enhances hippocampal CA1 LTP, and inhibition of ROS significantly impairs hippocampal CA1 LTP [58-62]. Metformin also significantly reduces pathology-associated reductions in LTP in animal models *in vivo*, indicating that learning and memory are linked to biostasis and aging deceleration, virus eradication, and human life creation via the induction of beneficial levels of cellular stress [63].

Lastly, cellular stress and AMPK activation also links the activation and mobilization of transposable elements (i.e. "jumping genes") with learning and memory and the creation of human life. Transposable elements (TEs) are DNA sequences first described by Nobel laureate Barbara McClintock that comprise nearly half of the human genome, are able to transpose or move from one genomic location to another, and have played an extensive role in human genome evolution [64-66]. Strikingly, McClintock also described in her Nobel Prize speech that a genome "shock" seemed to promote TE activation and mobilization [66]. As I first proposed in my recently published paper, this "shock" is the same "shock" that HIV cure researchers are using during the "shock and kill" approach to reactivate latent HIV-1 to potentially effectuate a cure [7]. Indeed, several forms of cellular stress, including heat shock and radiation, have been convincingly shown to activate and enhance TE mobilization in several model organisms and in human cells [67-69]. This same "shock" McClintock referred to, mediated by increases in ROS, Ca2+, and/or an AMP(ADP)/ATP ratio, etc. is also what leads to the creation of human life, as the antibiotic ionomycin activates AMPK, promotes TE activation, and induces human oocyte activation [32,48,70]. LINE-1 (L1), a member of the retrotransposon class of TEs, is active and capable of mobilization in human oocytes, human sperm, and in human neural progenitor cells [71-73]. Inhibition of L1 impairs both oocyte maturation *in vitro* and long-term memory formation *in vivo* in mice [74,75]. L1 has also been detected in the human brain and is capable of mobilization in human neurons [73]. As noted above, AMPK is critical for oocyte maturation and metformin promotes hippocampal neurogenesis and spatial memory formation [45,76]. The landmark initial sequencing of the human genome also noted that both telomerase and RAG1 (promotes DNA cleavage and transposition in human cells) are derived from TEs [65]. Because metformin activates both telomerase and RAG1 via AMPK, it is likely that cellular stress-induced AMPK activation facilitates beneficial TE activation and mobilization (i.e. learning and memory associated with L1 mobilization), potentially linking human genome evolution and the creation of human life with hippocampal LTP, biostasis/decelerated aging, and virus eradication [77,78].

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Sincerely,

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1. Finley J. Alteration of splice site selection in the LMNA gene and inhibition of progerin production via AMPK activation. Med Hypotheses. 2014 Nov;83(5):580-7. https://www.ncbi.nlm.nih.gov/pubmed/25216752

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7. Finley J. Transposable elements, placental development, and oocyte activation: Cellular stress and AMPK links jumping genes with the creation of human life. Med Hypotheses. Under Review.

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Facilitation of hippocampal long-term potentiation and reactivation of latent HIV-1 via AMPK activation: Common mechanism of action linking learning, memory, and the potential eradication of HIV-1

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Rapamycin
Oocyte
Acrosome
Telomerase
Transposable element (TE)
Retrotransposon
LINE-1 (L1)
Stress

ABSTRACT

Learning and memory are generally considered the behavioral correlates of long-term potentiation (LTP), a form of synaptic plasticity associated with a persistent and long-lasting increase in synaptic strength. Repetitive stimulation of excitatory synapses in the hippocampal CA1 region leads to release and binding of glutamate to the glutamate receptors AMPAR and NMDAR located on pyramidal neurons. Activation of AMPARs facilitates Na^+ influx, postsynaptic depolarization, NMDAR-mediated Ca^{2+} influx, and activation of several intracellular mechanisms that characterize LTP, including increased AMPAR synthesis, ROS production, and ER Ca^{2+} release. BDNF-TrkB receptor signaling, which increases intracellular Ca^{2+} levels via PLCγ1-mediated ER Ca^{2+} release, also plays an important role in facilitating hippocampal CA1 LTP. Interestingly, the cellular mechanisms that characterize LTP are strikingly similar to signaling pathways that underlie reactivation of latent HIV-1 reservoirs. Known as the "shock and kill" approach, reactivation of latent HIV-1, particularly in $CD4^+$ memory T cells, is currently being pursued to potentially eradicate HIV-1. Indeed, AMPARs, NMDARs, and TrkB receptors have been found on and promote T cell activation and BDNF has been shown to reactivate latent HIV-1 in human macrophages. Additionally, latent HIV-1 reactivation via T cell receptor activation (a positive control in HIV-1 latency studies) involves PLCγ1-mediated increases in intracellular Ca^{2+}, an increase in ROS levels, and activation of kinases and transcription factors that are also critical for LTP. Furthermore, PMA, also used as a positive control along with ionomycin in HIV-1 latency studies, has been shown to enhance hippocampal CA1 LTP. AMPK, an evolutionarily conserved kinase activated by increases in intracellular Ca^{2+}, ROS, and/or AMP/ATP ratio increases improves lifespan and healthspan in several model organisms and is essential for T cell activation. Knockdown of AMPK also significantly inhibits HIV-1 replication. AMPK has been found localized in hippocampal CA1 dendrites and glutamate, NMDA, KCl, ionomycin, and BDNF have each been shown to induce AMPK activation in neurons. AMPK activation also increases synthesis and membrane insertion of AMPARs. Because both T cell activation and LTP are dependent on intracellular Ca^{2+} increases and because inhibition of ROS significantly inhibits hippocampal CA1 LTP and T cell activation, it is our hypothesis that AMPK links latent HIV-1 reactivation with hippocampal LTP, learning, and memory. We also propose that compounds that enhance or promote LTP and reactivate latent HIV-1 (e.g. PMA, ionomycin, resveratrol, metformin, etc.) either alone or in combination likely do so via AMPK activation.

Introduction

Synaptic plasticity refers to an activity- or experience-dependent change in the strength of synaptic transmission and is considered a primary neural mechanism that underlies learning and memory [1]. The mammalian hippocampus, a seahorse-shaped structure located in the medial temporal lobe of the human brain, has been a focus of intense investigation due to its role in facilitating and promoting memory consolidation and learning [2]. The hippocampus is particularly vulnerable to morphological defects and synaptic pathology associated with neurodegenerative disorders characterized by progressive memory loss, such as Alzheimer's disease [3,4]. Similar to the hippocampus of the human brain, the rodent hippocampus, a well-studied model used to investigate mechanisms that delineate synaptic plasticity, is comprised of three consecutive synaptic pathways (perforant path-dentate gyrus, mossy fiber-CA3, and Schaffer collateral-CA1) that are subjected to various forms of stimulation to determine and analyze the degree of synaptic modification [5,6]. However, it is the Schaffer collateral-CA1

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excitatory synapse (herein referred to as CA1 synapses) that has generated increased interest in recent years due to accumulating evidence that high frequency stimulation of CA1 synapses leads to a long–lasting increase in synaptic strength, known as long-term potentiation (LTP) [6]. LTP at hippocampal CA1 synapses has been shown to be dependent on post-synaptic calcium (Ca^{2+}) influx mediated by activated N-methyl-d-aspartate (NMDA) receptors and is thought to be linked to the formation of associative memories, a form of declarative memory characterized by learning and the ability to recall the relationship between unrelated items [1,6].

HIV-1, the most common and pathogenic strain of the human immunodeficiency virus (HIV), is the cause of the majority of global HIV infections associated with the onset of acquired immunodeficiency syndrome (AIDS), a typically fatal condition characterized by a deficiency in cell-mediated immunity precipitated by a significant decrease in the number of $CD4^+$ T cells [7–9]. CD4 + T cells are a subset of T helper cells that express the surface protein CD4 and play a critical role in adaptive immunity and orchestrating an effective immune response to viruses and pathogens [9]. Although HIV-1 replication can be inhibited by the use of highly active anti-retroviral therapy (HAART), the establishment of a durable and long-lasting reservoir of latently-infected but replication-competent cells, notably in $CD4^+$ memory T cells, presents a significant barrier towards the effectuation of a cure [7,10]. As HAART targets replicating viruses capable of inducing viral gene expression and T cells latently infected with HIV-1 may undergo homeostatic proliferation without inducing latent HIV-1 reactivation, viral cytopathic or immune-mediated lysis of infected cells may be inefficient in reducing the size of the reservoir [7,11,12]. A method known as the "shock and kill" approach among HIV-1 cure researchers is currently being pursued to potentially eradicate HIV-1 via selective reactivation ("shock") and destruction ("kill") of latent HIV-1 reservoirs using compounds that target various pathways that play integral roles in reactivation of the virus (e.g. transcription factor binding, nucleosome remodeling, epigenetic modifications) [7,13]. However, recent evidence indicates that such compounds will likely be clinically ineffective if they are incapable of reactivating the virus via T cell activation, a method through which positive controls such as anti-CD3/anti-CD28 monoclonal antibodies (mAbs) and phorbol 12-myristate 13-acetate (PMA) combined with ionomycin (Io) reactivate the virus in latency reversal studies [7,14]. Similar to the requirement of an increase in intracellular Ca^{2+} levels in hippocampal pyramidal neurons for the induction of LTP, T cell activation-induced latent HIV-1 reactivation by anti-CD3/anti-CD28 mAbs and PMA/Io are also dependent on intracellular Ca^{2+} increases [6,7]. Additionally, as discussed below, similar intracellular signaling mechanisms and mediators also characterize both LTP and latent HIV-1 reactivation, indicating that two seemingly disparate cellular phenomena may be linked by a common mechanism.

The hypothesis

We propose the novel hypothesis that induction of cellular stress (e.g. increase in intracellular Ca^{2+}concentration, reactive oxygen species [ROS] generation, increase in AMP(ADP)/ATP ratio, etc.) and subsequent activation of 5′ adenosine monophosphate-activated protein kinase (AMPK) represents a common mechanism of action linking latent HIV-1 reactivation (i.e. the "shock and kill" approach) with hippocampal LTP, learning, and memory (i.e. the "shock and live" approach). Both T cell activation and physiological induction of hippocampal CA1 LTP are dependent on an increase in intracellular Ca^{2+} levels whereas a reduction in the levels of ROS significantly inhibits LTP and T cell activation. NMDARs, AMPARs, and TrkB receptors located on pyramidal neurons are well-studied critical mediators of LTP and are also present on and modulate T cell activation. Mitochondria translocate to the immunological synapse on T cell activation and to the postsynaptic membrane on synaptic stimulation, functioning as Ca^{2+} buffers and facilitating increased energy requirements for both cell types. Intracellular Ca^{2+} increases also potentiate ROS production from mitochondria and NADPH oxidases and increased ROS levels in turn promote Ca^{2+} release from ER Ca^{2+} stores, forming a feed-back loop critical for initiating and propagating similar signaling mechanisms that facilitate both latent HIV-1 reactivation and hippocampal LTP (e.g. PKC, ERK, Egr1, NF-kB, AP-1, NFAT, CREB, etc). Interestingly, activation of TCRs and TrkB receptors leads to PLCγ1-mediated increases in intracellular Ca^{2+}, with TCR activation used as a positive control to reactivate latent HIV-1 and BDNF-TrkB signaling reactivating latent HIV-1 in human macrophages. Additionally, resveratrol, a naturally-occurring phytoalexin, reactivates latent HIV-1 in T cell models and induces AMPAR synthesis and membrane insertion in hippocampal neurons in an AMPK-dependent manner while glutamate, NMDA, KCl, ionomycin, and BDNF also induce AMPK activation in neurons. Moreover, AMPK has been found localized in hippocampal CA1 dendrites and promotes mitochondrial biogenesis and functionality, CaMKK2 (an upstream AMPK activator) is upregulated on T cell activation and promotes LTP, knockdown of CaMKK2 or AMPK significantly inhibits HIV-1 replication, and PMA and/or ionomycin activates AMPK, enhances LTP, and promotes latent HIV-1 reactivation. Because AMPK activation improves lifespan and healthspan in several model organisms, is induced by intracellular increases in Ca^{2+}, ROS, and/or AMP(ADP)/ATP ratio increases, and AMPK activators including metformin ameliorates pathology-associated learning and memory deficits, activation of AMPK links LTP, learning, and memory with the potential eradication of HIV-1 via latent HIV-1 reactivation.

Evaluation and discussion of the hypothesis

AMPK activation is critical for T cell activation and latent HIV-1 reactivation

AMPK is an evolutionarily conserved fuel sensor present in various tissues including brain and cells of the immune system and functions primarily to regulate energy homeostasis by inhibiting anabolic pathways and stimulating catabolic pathways [15–18]. AMPK is activated by the induction of cellular stress, with an increase in the AMP(ADP)/ATP ratio or intracellular Ca^{2+} increases leading to AMPK phosphorylation and activation by the upstream kinases liver kinase B1 (LKB1) and calcium/calmodulin-dependent protein kinase kinase-β (CaMKKβ or CaMKK2), respectively [15,19,20]. AMPK is also responsive to alterations in cellular redox status, as increases in reactive oxygen species (ROS) induce AMPK phosphorylation and activation [21]. AMPK activation also enhances mitochondrial biogenesis, functionality, and oxidative phosphorylation through upregulation of mitochondrial transcription factor A (TFAM), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), and nuclear respiratory factors 1 and 2 (NRF-1/NRF-2) [15,22–24]. Autophagy, a cellular process critical for the removal and/or recycling of damaged proteins and organelles, is also induced by both AMPK and increases in intracellular Ca^{2+} levels [25,26]. Many structurally diverse compounds have been shown to induce AMPK activation, including the biguanide metformin, the adenosine monophosphate (AMP) analog 5-aminoimidazole-4-carboxamide-1-beta-4-ribofuranoside (AICAR), as well as various naturally-occurring compounds (e.g. resveratrol, bryostatin-1, rapamycin, tetrahydroxystilbene glucoside, etc.) [15,27–30].

As noted above, the establishment of proviral latency in viral reservoirs including $CD4^+$ memory T cells presents a significant barrier towards eradication of HIV-1. Although certain compounds have demonstrated latency-reversing effects by targeting mechanisms that promote proviral transcription, the most efficient and consistent means by which latent HIV-1 is reactivated is through full T cell activation via anti-CD3/anti-CD28 mAbs or the combinatorial use of PMA/Io, often used as controls in latency reversal studies [7,13,31]. Although PMA/Io induces T cell activation without engaging the CD3/T cell receptor

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(TCR) and CD28 co-receptor, the downstream signaling mechanisms that are activated are similar to TCR activation and are dependent on intracellular Ca^{2+} increases [31,32]. Briefly, TCR engagement initiates a signaling cascade that leads to the activation of phospholipase C-gamma 1 (PLCγ1), a phospholipid-cleaving enzyme that hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) to produce diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3). IP3 promotes ER Ca^{2+} release via binding to IP3 receptor type 1 (IP3R1) located on ER membranes, which also induces Ca^{2+} influx mediated by plasma membrane-associated CRAC channels [7,33]. DAG binds to and activates different isoforms of PKC (e.g. PKCθ), a kinase that plays an important role in the induction of transcription factors critical for T cell activation, including NFAT, AP-1, and NF-kB [7,34,35]. Other cellular mediators that are activated in response to TCR stimulation include ERK1/2, CaMKK2, Egr-1, and CREB [36–39].

Recent evidence also strongly suggests that similar to increases in cellular Ca^{2+} levels, an increase in intracellular levels of ROS plays a critical role in T cell activation. Ca^{2+} and ROS have been shown to engage in a positive feedback-mechanism wherein Ca^{2+} potentiates ROS production from ROS-generating organelles and enzymes and ROS in turn promotes ER Ca^{2+} release mediated by ryanodine receptors (RYRs) and IP3Rs [40]. NADPH oxidase-derived ROS has recently been shown to be essential in promoting $CD4^+$ T cell activation and the Ca^{2+} buffering capacity and translocation of mitochondria to the immunological synapse on T cell activation, inducing the early activation marker CD69 and IL-2 transcription, suggests that Ca^{2+}-induced, mitochondrial-derived ROS also enhances T cell activation [41–43]. Moreover, PMA and Io have been shown in independent studies to increase the levels of cellular ROS [44,45]. Additionally, increases in ROS and Ca^{2+} promote the induction of autophagy, TCR engagement activates autophagy in $CD4^+$ T cells, and autophagy blockade significantly inhibits T cell activation, indicating that an interplay between ROS and Ca^{2+} likely plays a pivotal role in T cell activation [26,46,47].

Sena et al. recently demonstrated that Ca^{2+}-induced mitochondrial ROS production was required for antigen-specific $CD4^+$ T cell activation *in vitro* as well as antigen-specific expansion of both $CD4^+$ and $CD8^+$ T cells *in vivo* [48]. The authors showed that mitochondrial metabolism was sufficient for supporting signaling mechanisms required for initial T cell activation. The addition of sodium pyruvate to T cells stimulated by anti-CD3/anti-CD28 mAbs restored the viability of T cells that had been previously activated in the absence of glucose, as evidenced by induction of IL-2 mRNA and upregulation of the T cell activation makers CD69 and CD25 [48]. Mitochondrial-derived ROS stimulated by Ca^{2+} influx was also necessary for $CD4^+$ T cell activation, as an extracellular Ca^{2+} chelator, a release-activated calcium (CRAC) channel inhibitor, and blockade of mitochondrial Ca^{2+} uptake inhibited mitochondrial ROS production whereas IL-2 induction was decreased by a mitochondrial-targeted antioxidant [48]. $CD4^+$ T cell activation was dependent on mitochondrial ROS derived from complex III of the electron transport chain, as mice with a conditional deletion of a gene encoding a complex III subunit necessary for ROS production had fewer activated but more naive $CD4^+$ T cells compared to control mice [48].

Because an increase in intracellular levels of ROS and Ca^{2+} are essential, if not indispensable, for efficient T cell activation and because AMPK, a kinase present in immune cells that improves lifespan, healthspan, and promotes mitochondrial biogenesis and functionality, is activated by both ROS and Ca^{2+}, it would be expected that AMPK would be a critical regulator of T cell activation and the immune response [49]. Indeed, Tamás et al. initially showed that the AMPKα1 isoform is present in both mouse and human T lymphocytes and AMPK is activated in response to TCR stimulation in a CaMKK2-dependent manner, a response that was also mimicked by ionomycin and thapsigargin through elevation of intracellular Ca^{2+} levels [50]. TCR triggering did not however increase the AMP/ATP ratio, indicating that AMPK activation via TCR triggering is not secondary to ATP depletion [50]. As noted above, activation of T cells via TCR stimulation involves PLCγ1-mediated production of IP3, which induces ER Ca^{2+} release and the formation of DAG. DAG in turn activates various PKC isoforms including PKCθ, an isoform that plays a crucial role in T cell activation by promoting the formation of the supramolecular activation complex and inducing the activation of the transcription factors NFAT, AP-1, and NF-kB [34,35]. Inhibition of PKCθ blocks T cell activation in Jurkat T cells, AMPK has been shown to activate PKCθ *in vitro*, and AMPK is activated in $CD8^+$ T cells *in vivo* during primary responses. AMPKα1 deletion also leads to a decrease in primary T cell responses to bacterial and viral infections *in vivo*, indicating that AMPK likely represents a central node in orchestrating efficient T cell activation and an effective immune response [18,51–54].

Additionally, Rao et al. showed that in mice with a conditional deletion of AMPKα1 in both $CD4^+$ and $CD8^+$ T cells (i.e. AMPK KO mice), T cell activation by PMA/Io or TCR triggering led to an increase in cell death compared to wild type (WT) cells *in vitro*, indicating that AMPK is critical for promoting efficient T cell activation [55]. *In vivo*, tumor weight and volume was higher in AMPK KO mice and T cell presence was significantly diminished in tumors. A higher percentage of $CD8^+$ T cells died in the tumor stroma and tumors in AMPK KO mice also experienced a 2-fold increase in $CD4^+$ T cell death compared to WT mice. Such evidence strongly suggests that AMPK activation enhances both T cell-mediated tumor immuno-surveillance and latent HIV-1 reactivation (see below) by promoting efficient T cell activation [55].

The most effective means through which latent HIV-1 is reactivated, facilitating immune system detection and destruction of the virus or virus-mediated cell death, is via T cell activation [31]. Candidate latency reversal compounds will thus likely prove to be clinically ineffective if they are incapable of inducing low-level T cell activation while avoiding global immune system stimulation. Increases in intracellular ROS and Ca^{2+} are critical for T cell activation and PMA and Io have both been shown in independent studies to increase Ca^{2+} and ROS levels [45,48,56–58]. Because AMPK is activated by increases in the levels of ROS, Ca^{2+}, and/or AMP(ADP)/ATP ratio increases, is activated by PMA, Io, and TCR stimulation, and is essential for T cell activation, it would be expected that AMPK would represent a common mechanism of action for compounds and methodologies that effectively reactivate latent HIV-1 [19–21,50,55,59]. Indeed, inhibition of PKCθ blocks Jukat T cell activation and inhibits HIV-1 replication in both $CD4^+$ T cells and peripheral blood lymphocytes while AMPK activates PKCθ *in vitro* [51–53,60,61]. Also, the induction of ROS itself, which activates AMPK, has been shown to reactivate HIV-1 in latently infected monocytes (U1) and lymphocytes (ACH-2) [62]. Preliminary data has also demonstrated that reactivation of latent HIV-1 in patient-derived $CD4^+$ T cells is significantly inhibited by N-acetylcysteine (NAC, a ROS scavenger) by ~55% and heat shock factor-1 (HSF1) inhibition by ~80% but is increased by an Nrf2 agonist, a master antioxidant transcription factor that is activated by increases in cellular ROS and by AMPK [63–65].

The induction of heat shock represents a particularly intriguing example of stress-induced latent HIV-1 reactivation. Heat shock (subjecting a cell to a higher temperature than the ideal body temperature) leads to induction of the heat shock response and activation of HSF1, an important regulator of heat shock proteins (HSPs) that promote repair or degradation of misfolded proteins [66]. Heat stress increases ROS production, promotes Nrf2 nuclear translocation, and activates AMPK whereas HSPs also respond to increases in intracellular Ca^{2+} or ROS [67–70]. Studies have shown that HSF1 is activated during T cell activation at fever-like temperatures (39.5 °C), culturing primary $CD4^+$ T lymphocytes at 39.5 °C increases HIV-1 replication 2–7 fold, and HIV-1 transcription is improved or inhibited by HSF1 overexpression or HSF1 knockout, respectively [71–73]. Hyperthermia also enhances HIV-1 reactivation in latently-infected cells in a HSP90-dependent manner, HSP90 promotes latent HIV-1 reactivation in $CD4^+$ T cells by

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enhancing transcription factor activity (e.g. NF-kB, NFAT, and STAT5), and HSP90 interacts with and maintains AMPK activity, indicating that stress-induced AMPK activation is likely a critical mediator in promoting latent HIV-1 reactivation [72,74–76].

Several structurally diverse compounds that activate AMPK have also been shown to reactivate latent HIV-1, further implicating cellular-stress induced AMPK activation as a common mechanism for HIV-1 reactivation. The phytoalexin resveratrol, for example, was recently shown, along with MG132 (proteasome inhibitor) and hemin (iron-containing porphyrin), to reactivate latent HIV-1 in a Jurkat T cell line [73]. Resveratrol, MG132, and hemin also activate AMPK [77–79]. Resveratrol-induced reactivation of HIV-1 in latently-infected ACH-2 cells, as evidenced by a dose dependent increase in viral p24 expression, was associated with an upregulation of Egr-1, a transcription factor that is also activated on TCR stimulation [80]. Resveratrol has also recently been shown to reactivate HIV-1 in latency lymphocyte cell models via activation of HSF1 and selective inhibition of HSF1 significantly reversed resveratrol-induced latent HIV-1 reactivation [191]. The HDAC inhibitor butyrate has been shown to reactivate latent HIV-1 in ACH-2 and U1 cells and trichostatin A-induced latent HIV reactivation was associated with a decrease in the pre-mRNA splicing activity of SRSF1 (or ASF/SF2). SRSF1 is a splicing factor that promotes HIV-1 latency through excessive splicing of the HIV-1 genome, preventing the production and nuclear export of unspliced genomic HIV-1 mRNA necessary for production of the viral p24 antigen [7,81,82]. Both butyrate and trichostatin A (TSA) have been shown to activate AMPK [83,84]. Intriguingly, TSA-induced reductions in SRSF1 activity was mediated by an increase in the activity of p32, a splicing-associated protein and endogenous inhibitor of SRSF1 that interacts with all mitochondrial mRNA species, is critical for mitochondrial biogenesis and functionality, constitutively associates with PKCθ *in vivo*, and translocates to the nucleus and colocalizes with PKCθ after PMA/TPA treatment [7,82,85,86].

As AMPK is critical for T cell activation, promotes PKCθ activation *in vitro*, and is activated by PMA/Io and TCR stimulation, it would also be expected that the biguanide metformin, a well-studied AMPK activator, would induce immuno-modulatory effects and enhance or promote latent HIV-1 reactivation. Metformin has been shown to enhance the tumor-infiltrating activities of $CD8^+$ T lymphocytes, increase the proliferation of splenocytes/lymphocytes in aged mice, and potentiate anti-CD3/anti-CD28 mAb-induced $CD4^+$ T cell activation (i.e. increased IL-2 production) [87–89]. Similar to TSA-induced latent HIV-1 reactivation, metformin has also been shown to decrease the mRNA and protein levels of the splicing factor SRSF1 in cells derived from patients with Hutchinson-Gilford progeria syndrome (HGPS), a disorder characterized by an accelerated aging phenotype induced by the excessive use of a cryptic splice site in the LMNA gene [90]. Interestingly, the endogenous SRSF1 inhibitor p32 is also critical for both rapamycin- and starvation-induced autophagy and autophagy blockade significantly inhibits T cell activation. Ca^{2+} and ROS independently promote autophagy induction and rapamycin-induced autophagy was shown to be Ca^{2+}-dependent. Rapamycin also induces AMPK activation *in vivo* in normal aged mice and metformin and rapamycin promote ULK1-mediated autophagy, indicating that both metformin and rapamycin may promote or enhance latent HIV-1 reactivation [25,26,29,46,47,91,92]. Introduction of human p32 to HIV-infected murine cells restores HIV replication (mouse p32 is inefficient in preventing SRSF1-induced HIV genome splicing and hence HIV replication is blocked in murine cells) and metformin decreases FAS (CD95) exon 6 exclusion (similar to T cell activation) in peripheral blood lymphocytes of diabetic patients, further indicating that AMPK activation also promotes latent HIV-1 reactivation by modulating alternative splicing [93–95].

Metformin has also been shown, when combined with the PKC modulator bryostatin-1, to promote latent HIV-1 reactivation in a monocytic (THP-89) cell line via AMPK-dependent phosphorylation of PKC [28]. In that same study, bryostatin-1 reactivated latent HIV-1 in a lymphocytic (J1.1) cell line and also induced AMPK activation, providing further evidence that chemically distinct compounds that reactivate latent HIV-1 may do so via cellular stress-induced AMPK activation [28]. In resting $CD4^+$ T cells isolated from HIV-1 + cART-treated aviremic patients, bryostatin-1 combined with the BET bromodomain inhibitor JQ1 led to reactivation of latent HIV-1 that was comparable to anti-CD3/anti-CD28 mAb stimulation as well as an increase in surface expression of the T cell activation maker CD69 in resting $CD4^+$ T cells from uninfected donors [96]. JQ1 has also recently been shown to activate AMPK via the induction of cellular stress and Laird et al. observed an increase in expression of the T cell activation markers CD69 and CD25 on latent HIV-1 reactivation with bryostatin-1 + JQ1, indicating that short-term and low level T cell activation mediated by AMPK, without inducing global T cell activation, is likely necessary to fully purge viral reservoirs [97,98]. Lastly, and perhaps most convincingly, a genome-scale siRNA screen for host factors that were required for HIV replication revealed that cellular knockdown of both *PRKAA1* (gene that encodes the catalytic subunit of AMPK) and *CAMKK2* (gene that encodes the upstream AMPK kinase CaMKK2) potently inhibited HIV infection in HeLa P4/5 cells at both 48 and 96 hr post-infection, corresponding to the stages of viral entry and all stages of infection from virus entry to release and spread, respectively [99]. siRNA-induced knockdown of *PRKAA1* and *CAMKK2* also inhibited subsequent infection of fresh HeLa P4/R5 cells by released virions. However, inhibition of HIV replication was restored by the expression of *PRKAA1* cDNA lacking the siRNA-targeted 3′UTR compared to an expression vector alone, indicating that AMPK is a critical mediator in facilitating HIV-1 replication [99].

Glutamate receptors and TrkB receptors are located on immune cells and modulate LTP, T cell activation, and latent HIV-1 reactivation

As previously discussed, the mammalian hippocampus has been a primary focus of intense investigation due to its role in facilitating learning and memory. The CA1 region of the hippocampus has been extensively studied due to accumulating evidence indicating that activity-dependent modification of hippocampal CA1 synapses is associated with memory formation *in vivo* [100–102]. Similar to the reactivation of latent HIV-1 reservoirs via T cell activation by anti-CD3/anti-CD28 mAbs or PMA/Io, the induction of hippocampal CA1 LTP, considered to be a cellular correlate of learning and memory, is also dependent on an increase in intracellular Ca^{2+} levels [31,103]. Induction of CA1 LTP involves presynaptic release of the excitatory neurotransmitter glutamate and subsequent binding of glutamate to the post-synaptic glutamate receptors α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) and NMDARs [103]. Glutamate binding to AMPARs facilitates Na^+ influx, leading to postsynaptic depolarization and subsequent expulsion of a magnesium ion that blocks the NMDAR, allowing Ca^{2+} influx through the NMDAR and induction of NMDAR-dependent CA1 LTP [103].

Following LTP induction, the activation of several Ca^{2+}-dependent signaling pathways within approximately the first hour leads to expression of LTP, a process characterized by synaptic alteration and an increase in the number, phosphorylation, and membrane insertion of AMPARs [6]. Although CaMKII, a Ca^{2+}-dependent kinase, has been shown to phosphorylate and increase the single-channel conductance of AMPARs, trafficking, lateral diffusion, and insertion of an increased number of AMPARs within the postsynaptic-density (PSD) facilitated by proteins including PSD-95 is considered to be the major mechanism of LTP expression at CA1 synapses [6,104]. The persistence and maintenance of LTP for more than 1–2 h is thought to be dependent on both local protein synthesis within dendrites and nuclear transcription [105]. Interestingly, the activation of Ca^{2+}-depenent signaling cascades that promote maintenance of LTP include several protein kinases and transcription factors that also mediate T cell activation and latent HIV-1

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reactivation, including PKC, PKA, ERK1/2, CaMKK2, Egr-1, NFAT, AP-1, NF-kB, and CREB [34–39,105–108].

Although LTP at CA1 synapses is dependent on NMDAR-mediated Ca^{2+} influx, intracellular Ca^{2+} increases during LTP induction is also facilitated by glutamate receptors that are not ion channels, including the metabotropic glutamate receptor mGluR5. Similar to TCR-mediated activation of T cells, glutamate binding to mGluR5 leads to the activation of a PLC isoform that hydrolyzes PIP2 to produce DAG, which activates PKC, and IP3, which promotes ER Ca^{2+} release via binding to IP3Rs located on ER membranes [33,109]. mGluR5 is extensively expressed in different areas of the brain including the hippocampus and the mGluR5 antagonist MPEP inhibits both the induction and late phases of LTP in the hippocampal CA1 region if applied before high-frequency tetanization, indicating that signaling mechanisms that induce intracellular Ca^{2+} release are also critical in facilitating LTP [110]. Furthermore, recent studies have provided compelling evidence that the activation of TrkB receptors by the neurotrophin brain-derived neurotrophic factor (BDNF) plays a critical role in promoting hippocampal CA1 LTP partly by inducing intracellular Ca^{2+} release [111]. Similar to the activation of mGluR5, BDNF binding to TrkB receptors induces a signaling cascade that is strikingly similar TCR-mediated activation of T cells, with BDNF-TrkB signaling leading to the activation of PLCγ1, which hydrolyzes PIP2 to produce DAG and IP3, with IP3 promoting ER Ca^{2+} release via binding to IP3Rs located on ER membranes and DAG activating PKC [111]. Other kinases and transcription factors that are activated on T cell activation and promote latent HIV-1 reactivation are also activated by BDNF-TrkB signaling, including ERK1/2, CREB, NFAT, and NF-kB [34–39,107,111,112]. Additionally, as NF-kB is a well-studied mediator of T cell activation and latent HIV-1 reactivation, BDNF has been shown to activate NF-kB, NF-kB activation increases BDNF levels in neurons, and PKC activates NF-kB. LTP-inducing high frequency stimulation also increases NF-kB subunit gene expression in the hippocampal CA1 region, NF-kB enhances dendritic spine and excitatory synapse density by regulating PSD-95 in hippocampal neurons, and mice lacking an NF-kB subunit show deficits in spatial learning, suggesting that NF-kB activation is critical for both T cell activation and hippocampal LTP [31,108,112–114]. TrkB receptors are extensively expressed in the hippocampus and BDNF-TrkB signaling has been shown to promote the formation of PSD-95-TrkB complexes. LTP at hippocampal CA1 synapses is also significantly inhibited by the BDNF scavenger TrkB-IgG or anti-TrkB antibodies, confirming that BDNF-TrkB signaling is critical in promoting hippocampal CA1 LTP [111,115].

Similar to the dependency of both T cell activation-induced latent HIV-1 reactivation and hippocampal LTP on intracellular Ca^{2+} increases, recent evidence indicates that increases in the levels of cellular ROS, which are necessary for T cell activation/latent HIV-1 reactivation, are also likely required for hippocampal CA1 LTP [48]. ROS and Ca^{2+} have been shown to engage in a positive feed back mechanism that involves Ca^{2+}-induced increases in ROS production from ROS-producing organelles and enzymes as well as ROS-induced potentiation of Ca^{2+} release from ER Ca^{2+} stores [40]. Activation of the BDNF-TrkB signaling pathway and mGluR5 activation leads to an IP3R-mediated release of Ca^{2+} from ER stores via activation of the PLC-PIP2-IP3-Ca^{2+} pathway, facilitating hippocampal CA1 LTP induced by NMDAR-mediated Ca^{2+} influx [109,111]. Moreover, BDNF has been shown to increase superoxide levels (O2–) in PC12h cells and increase NADPH oxidase-derived ROS in cortical neurons while mGluR5 activation increases mitochondrial ROS in an IP3-dependent manner in neurons, indicating that an interplay between ROS and Ca^{2+} plays a pivotal role in neuronal functioning [116–118]. ROS also potentiates Ca^{2+} release by ER-associated IP3Rs and RYRs and simultaneous inhibition of both receptors prevents tetanus-induced hippocampal CA1 LTP, indicating that IP3R- and RYR-mediated Ca^{2+} release play essential roles in facilitating LTP [119–121]. Interestingly, PMA and Io both increase intracellular ROS levels and PMA enhances hippocampal CA1 LTP in an NMDAR-dependent manner [31,44,45,122]. Also, similar to mitochondrial translocation to the immunological synapse on T cell activation, glutamate binding to and activation of NMDARs leads to mitochondrial recruitment to synapses in hippocampal neurons via NMDAR-mediated Ca^{2+} influx [123]. The induction of autophagy by ROS and Ca^{2+} also links T cell activation with synaptic plasticity, as autophagy blockade significantly inhibits T cell activation, stimulation of rat hippocampal neurons with potassium chloride (KCl) induces NMDAR-dependent autophagy, and environmental enrichment increases autophagy signaling and BDNF expression in the rat hippocampus [26,46,47,124,125].

ROS production may play as important of a role as intracellular Ca^{2+} increases in T cell activation, latent HIV-1 reactivation, and hippocampal CA1 LTP, as ROS is necessary for T cell activation, induces latent HIV-1 reactivation, and inhibition of ROS significantly inhibits reactivation of latent HIV-1 in patient-derived $CD4^+$ T cells in preliminary studies [48,62,63]. A number of studies have indicated that ROS is also required for the induction of hippocampal NMDAR-dependent LTP. Bindokas et al. demonstrated that treatment of rat hippocampal neurons with AMPA, NMDA, and kainic acid, ligands for the AMPAR, NMDAR, and kainate receptors (a glutamate receptor), respectively, led to an increase in superoxide (O2–) generation, with mitochondria being the major source of O2– under stimulated conditions [126]. Incubation of hippocampal slices with manganese porphyrin compounds that mimic superoxide dismutase (SOD) prevented the induction of hippocampal CA1 LTP by high-frequency stimulation while CA1 LTP was also inhibited in transgenic mice that overexpressed extracellular SOD [127,128]. Strikingly, treatment of hippocampal slices with a superoxide-generating system induces long-lasting potentiation of synaptic transmission in the hippocampal CA1 region that is occluded by HFS-induced LTP and is dependent on an increase in PKC activity, a kinase that is also activated by TCR, BDNF-TrkB, and GluR5 signaling and plays a critical role in promoting T cell activation, latent HIV-1 reactivation, and hippocampal CA1 LTP [31,33,109,111,129].

Similar to T cell activation, sources for ROS production involved in hippocampal CA1 LTP include mitochondria and NADPH oxidase. Strong Ca^{2+} entry induced by HFS stimulation in rat CA1/CA3 hippocampal neurons leads to an increase in O2– production by mitochondria located near plasmalemmal sites of Ca^{2+} influx. PKC activity also increased in response to superoxide production but was inhibited by ROS scavengers, suggesting that an increase in Ca^{2+}-induced mitochondrial respiration enhances O2– production and modulates downstream kinases important for hippocampal CA1 LTP [130]. Strong depolarization-induced Ca^{2+} entry in hippocampal neurons was shown to significantly increase mitochondrial-derived O2– and promote the elevation of phosphorylated CREB (pCREB), a transcription factor critical for hippocampal CA1 LTP that is also activated during T cell activation. Inhibition of mitochondrial Ca^{2+} uptake and respiration however blocked O2– production and depressed the elevation of pCREB [38,131]. Components of the O2– producing enzyme NADPH oxidase have also been found localized in dendrites in mouse hippocampal neurons and stimulation of hippocampal slices by the phorbol ester PMA induced translocation of NADPH oxidase proteins to the plasma membrane and increased the production of O2– [132]. PMA/Io is also used extensively as a positive control in HIV-1 latency reversal studies, ionomycin induces NADPH oxidase-derived ROS, and PMA enhances hippocampal CA1 LTP in an NMDAR-dependent manner, implicating ROS as an important mediator of T cell activation/latent HIV-1 reactivation and CA1 LTP [31,45,122]. Kishida et al. also demonstrated that treatment of mouse hippocampal slices with NMDA led to an NMDAR-dependent activation of the kinase ERK in area CA1 that was blocked by several antioxidants including SOD and the SOD mimetic MnTBAP. The NADPH oxidase inhibitor diphenylene iodonium also blocked the NMDAR-dependent activation of ERK, indicating that NADPH oxidase-derived ROS may be required for ERK activation in area CA1 [133].

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Although T cell activation-induced latent HIV-1 reactivation and hippocampal CA1 LTP are seemingly disparate physiological processes, their dependency on increases in intracellular Ca^{2+} and ROS levels indicates that these processes may be linked by the activation of AMPK, a master metabolic regulator that is activated by increases in intracellular Ca^{2+}, ROS, and/or AMP(ADP)/ATP ratio increases [19–21]. TCR-induced activation of T cells leads to the activation of AMPK in a CaMKK2-dependent manner and loss of CaMKK2 inhibits CREB activation, hippocampal CA1 late LTP, and spatial memory formation in CaMKK2-null mutant mice [50,106]. Knockdown of CaMKK2 and AMPK significantly inhibits HIV-1 replication, AMPKα1 deletion leads to a decrease in primary T cell responses to bacterial and viral infections *in vivo*, and TCR- or PMA/Io-induced T cell activation of $CD4^+$ and $CD8^+$ T cells with a conditional deletion of AMPKα1 leads to increased cell death [18,55,99]. PMA and Io both activate AMPK while PMA induces an increase in O2– in hippocampal neurons, induces autophagy, activates ERK, and enhances hippocampal CA1 LTP in an NMDAR-dependent manner [31,50,59,122,132]. Autophagy, which is induced by both AMPK and IP3-mediated increases in intracellular Ca^{2+} levels, is also essential for T cell activation and stimulation of hippocampal neurons and environmental enrichment increases autophagy signaling [25,26,47,124,125].

Because T cell activation/latent HIV-1 reactivation and hippocampal CA1 LTP share many common mediators and mechanisms that are associated with AMPK activation, it would be expected that neuronal stimulation or depolarization would lead to AMPK activation. As discussed in further detail in the next section, AMPK has been found localized in dendrites in hippocampal CA1 neurons and is activated by structurally diverse compounds that induce cellular stress, including metformin, 2-deoxy-D-glucose (2DG), and the AMP mimetic AICAR [134]. AMPK is also activated in neurons by the primary excitatory amino acid glutamate as well as NMDA, KCl, ionomycin, and A23187 (a Ca^{2+} ionophore), suggesting the novel observation that both T cell activation/latent HIV-1 reactivation and hippocampal CA1 LTP are similarly orchestrated by cellular-stress induced AMPK activation [135,136]. mGluR5s, AMPARs, NMDARs, and TrkB receptors have also been found on T cells and modulate mechanisms associated with T cell activation. mGluR5s for example are expressed in resting and activated T lymphocytes and in Jurkat T cells and mGluR5 activation in lymphocytes leads to activation of adenylate cyclase (an enzyme that catalyzes the conversion of ATP to cAMP on T cell activation) and an increase in intracellular Ca^{2+} levels in Jurkat T cells. Activation of mGluR1, a member of the same class of mGluRs as mGluR5, also upregulated the immediate early genes *c-fos* and *c-jun* (genes that are rapidly expressed on T cell activation) and mGluR5 activation has been shown to induce AMPK phosphorylation and activation [137–139]. Human lymphocytes also express AMPARs and the application of glutamate or AMPA prior to stimulation with either PHA or anti-CD3 mAbs potentiated the increase of intracellular Ca^{2+} [140]. Normal human T cells have also been shown to express high levels of the AMPA receptor subtype 3 (GluR3) and glutamate, in the absence of any other molecule, triggers T cell functions normally performed by activated T cells, including adhesion to laminin and fibronectin and chemotactic migration, effects that were also mimicked by the AMPAR agonist AMPA [141]. Interestingly, a Ca^{2+}-permeable AMPAR generated by a lack of mRNA editing has been found in mouse spleen cells and an AMPAR antagonist was shown to suppress $CD4^+$ T cell proliferation [142].

Furthermore, as glutamate and NMDA activates AMPK in neurons, AMPK is critical for T cell activation and HIV-1 replication, and T cell activation by anti-CD3/anti-CD28 mAbs or PMA/Io efficiently induces latent HIV-1 reactivation, NMDARs are also found on T cells and regulate mechanisms associated with T cell activation [18,31,55,99,135]. Incubation of human lymphocytes with the NMDAR agonist NMDA leads to a significant increase in intracellular Ca^{2+} and ROS levels, with NMDA-induced ROS production in T cells resembling the response of neuronal cells to NMDA [143]. ROS formation in human lymphocytes is also inhibited by the NMDAR antagonist MK-801 and PMA, which activates AMPK and enhances hippocampal CA1 LTP, also increased ROS content in lymphocytes, suggesting that in addition to functioning as a DAG analogue and activating PKC, PMA may promote T cell activation/latent HIV-1 reactivation and CA1 LTP by inducing ROS production [59,122,144]. The NMDAR may also regulate T cell activation by modulating Ca^{2+} influx, as the NMDAR antagonist MK-801 inhibits Ca^{2+} influx induced by thapsigargin in T cells [145]. Importantly, Miglio et al. demonstrated that the NMDAR is functionally active in controlling T cell activation, as D-AP5 and MK-801 (competitive and non-competitive NMDAR antagonists, respectively) both inhibited PHA-induced T cell proliferation as well as the expression of CD25, a plasma membrane marker of T cell activation [146].

Additionally, because TCR stimulation and BDNF-TrkB signaling both activate PLCγ1, leading to the production of DAG and IP3, it would be expected that BDNF-TrkB signaling would also regulate T cell function. Peripheral T cells have been found to express TrkB, BDNF binding to TrkB imparts increased survival on T cell activation, and activated human $CD4^+$ T cells also increase BDNF production [147,148]. Recent studies have shown that repetitive transcranial magnetic stimulation (rTMS), which induces neuronal LTP, simultaneously improves BDNF-TrkB signaling in both neurons and lymphocytes *in vivo*. In rats, 5 Hz of rTMS increased the affinity of BDNF for and phosphorylation of TrkB, increased PLCγ1 recruitment to TrkB, increased the association between activated TrkB and the NMDAR, and increased the downstream activities of ERK2 and PI3K in lymphocytes and in the prefrontal cortex [149]. In normal human subjects, 5d of rTMS to the motor cortex also correlated with increased BDNF-TrkB signaling and TrkB-NMDAR association in lymphocytes, findings that were later recapitulated in patients with Parkinson's disease, suggesting that a common mechanism links T cell activation and LTP [149,150]. Indeed, TCR activation-induced latent HIV-1 reactivation is dependent on activation of PLCγ1 and promotion of hippocampal CA1 LTP by BDNF-TrkB signaling has been shown to be dependent on PLCγ1. Mutation of the PLCγ1-binding sites of TrkB in mice blocks early and late phases of hippocampal CA1 LTP, concurrent blockade of pre- and postsynaptic PLCγ1 signaling and IP3 production significantly inhibits hippocampal CA1 LTP, and peptide sequestering of PLCγ1 in rat hippocampal neurons significantly impairs long-term memory in the Morris water maze task [151–153]. Furthermore, because TCR stimulation and PMA/Io activate AMPK and induce T cell activation-induced latent HIV-1 reactivation and because AMPK is essential for T cell activation and HIV-1 replication, it would also be expected that BDNF-TrkB signaling would activate AMPK and promote latent HIV-1 reactivation. Indeed, voluntary exercise enhanced learning and memory performance in rats that was associated with an increase in the mRNA levels of both AMPK and BDNF. BDNF blocking inhibited exercise-mediated enhancement in spatial learning, the association between learning speed and the levels of AMPK mRNAs, and the exercise-induced increase in AMPK mRNA levels, suggesting that BDNF promotes AMPK activation in hippocampal neurons [154].

Lastly, neurotrophins, including BDNF, have recently been shown to induce latent HIV-1 reactivation in human primary macrophages. Using primary human monocyte-derived macrophages from healthy donors that had been infected with the CCR5-dependent isolate HIV-1 $_{Ba-L}$, Souza et al. showed that both BDNF and nerve growth factor (NGF) stimulated virus replication and amplified HIV-1 production with similar potencies [155]. Similar to BDNF-TrkB signaling, NGF binds to the TrkA receptor, leading to PLCγ1-mediated activation of DAG, which activates PKC, and IP3, which induces IP3R-mediated Ca^{2+} release. NGF-induced HIV replication was prevented by an intracellular Ca^{2+} chelator as well as inhibitors of NF-kB, ERK, PKC, and PLCγ1, indicating that neurotrophin stimulation of HIV-1 replication involves PLCγ1 activation, mobilization of Ca^{2+} from ER stores, and PKC-mediated activation of ERK and NF-kB, mediators that are also critical for promoting hippocampal CA1 LTP [155].

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AMPK activation increases AMPAR synthesis and enhances LTP

Because hippocampal CA1 LTP and T cell activation/latent HIV-1 reactivation are dependent on an increase in intracellular Ca^{2+} levels and ROS and because AMPK is activated by ROS and Ca^{2+} increases, is critical for T cell activation and HIV-1 replication, and is found in hippocampal CA1 neurons, it is likely that AMPK activation plays a beneficial role in promoting hippocampal LTP. mGluR5 activation and BDNF have been shown to activate AMPK and enhance hippocampal CA1 LTP whereas loss of CaMKK2 (an upstream AMPK kinase) inhibits hippocampal CA1 late LTP [106,110,115,139,154]. PMA also enhances hippocampal CA1 LTP in an NMDAR-dependent manner, reactivates latent HIV-1 when combined with Io (Io also activates AMPK), and activates AMPK [31,50,59,122]. Additionally, Potter et al. showed that the phosphorylated/activated form of AMPK is present in both dendrites and cell bodies of mouse hippocampal CA1 pyramidal neurons and is activated by structurally diverse compounds including metformin, 2-deoxy-D-glucose (2DG), and the AMP mimetic AICAR [134]. AMPK is also activated in cortical and hippocampal neurons by compounds that are necessary for or enhance hippocampal CA1 LTP, including glutamate, NMDA, ionomycin, KCl, and A23187 [135,136].

Perhaps most convincingly, however, is the recent finding that AMPK activation increases synthesis and the level of surface AMPARs in hippocampal neurons, a process that is generally considered the most important component for the expression of LTP [77]. Incubation of cortical neurons with resveratrol significantly increased the amount of the AMPAR subunit GluA1 (a subunit essential for hippocampal LTP), synaptic AMPARs, and the levels of surface AMPARs. Intraperitoneal injection of resveratrol in mice increased AMPAR expression in the prefrontal cortex, hippocampus, and cerebellum compared to controls, confirming resveratrol's *in vivo* effect on AMPAR expression [77]. Resveratrol also increased AMPAR-mediated miniature excitatory postsynaptic currents (mEPSCs) in cultured hippocampal neurons. The resveratrol-mediated increase in AMPAR expression was dependent on protein synthesis but was SIRT1-independent (thought to be a direct target of resveratrol), as resveratrol increased GluA1 expression and synaptic accumulation in cortical and hippocampal neurons in the presence of EX527 (SIRT1 inhibitor) or siRNA-mediated knockdown of SIRT1, respectively [77]. Resveratrol also activated AMPK in neurons, the temporal profile of AMPK activation matched the changes in AMPAR expression, compound C (an AMPK inhibitor) and STO-609 (a CaMKK2 inhibitor) abolished the increases in AMPAR abundance and AMPK activation, and resveratrol-induced increases in GluA1 synaptic accumulation were completely abolished in hippocampal neurons via overexpression of a kinase dead form of the AMPKα1 subunit, indicating that resveratrol-mediated increases in AMPAR expression are AMPK-dependent [77].

The eIF4F translation initiation complex was also shown to be necessary for resveratrol-induced increases in AMPAR expression and AICAR significantly increased the total amount of GluA1 which was blocked by protein synthesis inhibitors and compound C, indicating that AICAR-induced increases in GluA1 expression is dependent on AMPK-mediated increases in GluA1 protein synthesis [77]. As AMPK is critical for both T cell activation and HIV-1 replication, resveratrol has been shown to reactivate latent HIV-1 in a Jurkat T cell line and induce HIV-1 reactivation in latently-infected ACH-2 cells, which was associated with an upregulation of Egr-1, a transcription factor that is upregulated on T cell activation and during hippocampal LTP [37,73,80,105]. Also, knockdown of brain-specific carnitine palmitoyltransferase 1C (CPT1C), an enzyme that promotes fatty acid beta-oxidation, leads to a significant reduction in the hippocampal synaptic levels of the AMPAR subunit GluA1, reductions in AMPAR-mediated mEPSCs, and reduced AMPK activation under basal conditions [156]. Metformin, which enhances latent HIV-1 reactivation when combined with the AMPK activator bryostatin-1, however increased CPT1C mRNA levels in MCF-7 cells whereas CPT1C mRNA levels were reduced in AMPK-deficient mouse embryonic fibroblasts, indicating that AMPK activation is a critical mediator for promoting AMPAR synthesis, CA1 LTP, and latent HIV-1 reactivation [28,157]. AMPK has also been shown to phosphorylate and activate CREB in mouse primary hippocampal neurons, a transcription factor that is activated on T cell activation and plays a critical role in CA1 LTP [38,105,158]. A beneficial effect of AMPK activation in learning and memory has also been demonstrated *in vivo*, with exercise and AICAR-treatment in chronically stressed mice enhancing alternation in the Y-maze test, reducing escape latency in the water maze test, promoting hippocampal neurogenesis, and enhancing the levels of phosphorylated/activated AMPK, BDNF, and PSD-95 in the hippocampus, effects that were inhibited by periodic injections of the AMPK inhibitor compound C [159]. PSD-95 is essential in facilitating membrane insertion of AMPARs within the postsynaptic-density and resveratrol increases the surface level expression of AMPARs in an AMPK-dependent manner, providing compelling evidence that AMPK activation may alleviate impairments in learning and memory *in vivo* [77,104]. Moreover, HFS-induced hippocampal synaptic LTP at the dentate gyrus *in vivo* in rats significantly increased activation of AMPK and induced inhibitory phosphorylation of GSK3β [160]. HFS-induced AMPK activation was associated with CaMKK2 activation and an increase in the AMP(ADP)/ATP ratio while inhibition of AMPK by compound C prevented HFS-induced early LTP induction, indicating that AMPK activation enhances hippocampal LTP, learning, and memory formation [160].

Structurally diverse compounds and mechanisms that promote LTP and latent HIV-1 reactivation likely do so via AMPK activation

Accumulating evidence strongly suggests that both hippocampal CA1 LTP and T cell activation-induced latent HIV-1 reactivation are dependent on increases in the levels of intracellular Ca^{2+} and ROS. Because AMPK is activated by the induction of cellular stress, mediated by increases in Ca^{2+}, ROS, and/or AMP(ADP)/ATP ratio increases, and because AMPK activation appears to play a pivotal role in promoting T cell activation, HIV-1 replication, and hippocampal LTP, structurally diverse compounds that beneficially modulate LTP and enhance latent HIV-1 reactivation likely do so via cellular stress-induced AMPK activation. For example, PMA and Io both increase intracellular levels of ROS and Ca^{2+}, activate AMPK, are used as positive controls to efficiently reactivate latent HIV-1, and PMA enhances hippocampal CA1 LTP [19,31,45,48,56–59,122]. Similarly, the phytoalexin resveratrol also reactivates latent HIV-1, increases synthesis and surface level expression of AMPARs and mEPSCs in neurons in an AMPK-dependent manner, and improves Aβ1-42-induced impairments in hippocampal CA1 LTP and spatial memory *in vivo* in rats, suggesting that AMPK activation may ameliorate memory impairments associated with neurodegenerative disorders such Alzheimer's disease [73,77,80,161]. Additionally, metformin enhances latent HIV-1 reactivation when combined with bryostatin-1, potentiates anti-CD3/anti-CD28 mAb-induced $CD4^+$ T cell activation, promotes the tumor-infiltrating activities of $CD8^+$ T lymphocytes, activates AMPK in hippocampal CA1 neurons, and increases CPT1C mRNA levels (CPT1C promotes AMPAR synaptic accumulation) [28,87,89,134,156,157]. Similar to the AMPK activator resveratrol, metformin has also been shown to significantly attenuate Aβ-mediated reductions in LTP-induced population spike amplitude and excitatory postsynaptic potential (EPSP) slope and ameliorate scopolamine-induced impairment in both the Morris water maze and passive avoidance tasks *in vivo* in rats [162,163]. In Aβ-treated human neural stem cells, metformin also normalized cell viability and significantly increased the mRNA levels of CREB, PGC-1a, NRF-1, and TFAM in an AMPK-dependent manner compared to Aβ-treatment alone [164]. Furthermore, in a randomized placebo-controlled clinical trial involving participants with amnestic mild cognitive impairment, metformin treatment led to an improvement in total recall of the Selective Reminding Test, a test designed to assess verbal learning and memory,

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again indicating that cognitive impairment and neurodegenerative disorders characterized by memory loss may be alleviated via AMPK activation [165].

Interestingly, epigenetic modifications that regulate chromatin structure also link hippocampal CA1 LTP, HIV-1 latency, and AMPK activation. G9a is a histone methyltransferase that induces transcriptional silencing of chromatin by catalyzing the dimethylated state of H3K9me2. G9a-catalzyed H3K9me2 is upregulated during HIV-1 latency and inhibition of G9a by the compound BIX-01294 significantly increases phosphorylation and activation of AMPK, reactivates latent HIV-1, and reinforces NMDAR-dependent early-LTP into late-LTP in hippocampal CA1 neurons [166–168]. The histone deacetylase (HDAC) inhibitors butyrate and trichostatin A (TSA) both activate AMPK, enhance induction of hippocampal CA1 LTP, and promote latent HIV-1 reactivation [81–84,169]. Also, TSA-induced latent HIV-1 reactivation is associated with a decrease in the pre-mRNA splicing activity of SRSF1 (ASF/SF2) by the splicing-associated protein p32, suggesting that similar activities in gene splicing factors may also link latent HIV-1 reactivation with LTP [82]. Indeed, neuronal depolarization or TSA-treated neurons decreased the recruitment efficiency of SRSF1 to nascent RNAs and promoted SRSF1 nuclear speckle accumulation whereas SRSF1 activity is down-regulated on T cell activation [170,171]. p32, an endogenous inhibitor of SRSF1, interacts with all mitochondrial mRNA species and is critical for mitochondrial functionality, binds to all PKC isoforms *in vitro*, is essential for starvation- and rapamycin-induced autophagy, and is translocated to the nucleus after treatment with PMA, implicating AMPK-induced down-regulation of the splicing factor SRSF1 and modulation of p32 as an additional component linking latent HIV-1 reactivation and hippocampal CA1 LTP [82,85,86,91]. The splicing activity of SRSF1 has also been shown to promote the utilization of a cryptic splice site in the LMNA gene and increase the production of the toxic protein progerin in Hutchinson-Gilford progeria syndrome (HGPS), a disorder characterized by an accelerated aging phenotype [90]. Metformin however has recently been shown to decrease the mRNA and protein levels of SRSF1, decrease progerin expression, activate AMPK, and alleviate accelerated aging defects in cells derived from HGPS patients, as we originally hypothesized and proposed in 2014 and 2015, indicating that AMPK links latent HIV-1 reactivation with both hippocampal CA1 LTP and HGPS [7,88,90,172].

Additionally, the orexigenic hormone ghrelin induces synaptic delivery of AMPARs in hippocampal CA1 neurons (similar to resveratrol), ghrelin receptor activation dramatically enhances hippocampal CA1 LTP expression, ghrelin significantly increases AMPK activation in neurons, and preliminary data has shown that ghrelin alleviates accelerated aging defects in cells derived from HGPS patients [188–190]. Moreover, rapamycin is dependent on p32 for autophagy induction, improves accelerated aging defects in HGPS cells by promoting autophagy-induced progerin reduction, restores BDNF-LTP and spatial memory in a mouse model of Down syndrome, and activates AMPK *in vivo* in normal aged mice [29,91,173,174]. A recent study also demonstrated that temsirolimus (a soluble ester of rapamycin) improved accelerated aging defects in HGPS cells but also increased the levels of ROS and superoxide within the first hour of treatment, implicating the novel observation that the alleviation of accelerated aging defects in HGPS cells by structurally diverse compounds involves the induction of cellular stress (e.g. ROS, Ca^{2+}, AMP(ADP)/ATP ratio increase, etc.), similar to latent HIV-1 reactivation and hippocampal CA1 LTP [175].

Furthermore, heat stress activates AMPK, HSF1 and HSP90 promote latent HIV-1 reactivation, HSP90 interacts with and maintains AMPK activity, and heat shock prevents scopolamine-induced suppression of hippocampal CA1 LTP, indicating that cellular-stress induced AMPK activation may represent a common mechanism linking seemingly disparate physiological and patho-physiological states [68,73,74,76,176]. Indeed, oocyte activation is a process that is indispensable for the creation of all human life and cellular stress-inducing compounds including the free radical-generating agent menadione and a resveratrol analog as well as heat stress induce oocyte meiotic resumption and maturation in an AMPK-dependent manner, processes that are critical for efficient oocyte activation [177–179]. Also, PMA has been shown to activate mouse oocytes (models for human oocytes), ionomycin is extensively used to activate human oocytes during *in vitro* fertilization procedures (producing normal healthy children), and metformin improves pregnancy rates during human *in vitro* fertilization procedures [180–182]. Additionally, the activated form of AMPK (phospho-Thr172-AMPK) has recently been identified in human spermatozoa, with prominent staining at the sperm acrosome [183]. The acrosome reaction is a process that involves the release of proteolytic enzymes that are essential for oocyte penetration and fertilization and PMA, ionomycin, and ROS each induce the acrosome reaction in human spermatozoa [184–186]. Such evidence not only further substantiates our hypothesis first published in 2016 implicating AMPK activation as a central node linking oocyte activation with latent HIV-1 reactivation, but also indicates that cellular stress-induced AMPK activation links oocyte activation with latent HIV-1 reactivation, the acrosome reaction in human sperm, improvement of accelerated aging defects in HGPS cells, and enhancement of hippocampal CA1 LTP [7,15,172].

Cellular stress may also promote and/or enhance memory formation by inducing the activation and mobility of transposable elements (TEs). TEs, also known as "jumping genes", are sequences of DNA first described by Nobel laureate Barbara McClintock that are able to transpose or move from one genomic location to another [192]. Such movement has been shown to alter the function of nearby genes, creating new gene regulatory networks or even facilitating new gene creation (e.g. exonization), thus significantly contributing to human genome evolution [192]. As McClintock first noted that transposition of TEs may result from a genome "shock", cellular stressors including heat stress and increases in ROS and Ca^{2+} have been shown to activate and/or induce transposition of TEs *in vitro* and *in vivo* [193–196].

A specific family of TEs known as Long Interspersed Nuclear Elements-1 (LINE-1s or L1s) have recently been shown to be active and capable of retrotransposition in human oocytes, human sperm, and in human neural progenitor cells located within the hippocampus [197–199]. ROS has also been shown to promote L1 retrotransposition in human neuroblastoma cells [196]. Exercise was shown to enhance L1 retrotransposition in the dentate gyrus of the hippocampus in mice and L1 expression and retrotransposition in the adult mouse hippocampus was reported to enable long-term memory formation [200,201]. Because AMPK is activated by cellular stressors that induce TE activation and transposition (heat stress, ROS, Ca^{2+}, etc) and because AMPK activation normalizes human neural stem cell viability in response to amyloid beta (Aβ) treatment, it is likely that AMPK activation also facilitates TE activation and/or transposition in appropriate cellular contexts [20,21,68,164].

Moreover, RAG1, an endonuclease that mediates DNA cleavage and is thought be derived from a TE, is critical for the assemblage of immunoglobulin and T cell receptor genes via V(D)J recombination and is phosphorylated and activated *in vivo* via metformin-induced AMPK activation [202–204]. RAG1 together with RAG2 is also capable of mediating DNA transposition *in vitro* [204]. Telomerase, a ribonucleoprotein with reverse transcriptase activity that plays a critical role in telomere maintenance, is also thought to be derived from TEs [202]. T cell activation, which is dependent on increases in ROS, Ca^{2+}, and AMPK activation has been shown to transiently increase telomerase activity, suggesting the novel observation that cellular stress and AMPK activation likely also enhances telomerase activation (e.g. via metformin) in appropriate cellular contexts [48,55,205].

Additionally, stress also induces TE activation and transposition in bacteria and increases in intracellular ROS and Ca^{2+} is associated with spore germination, aerial hyphae formation, and antibiotic production in the medicinally valuable bacterial genus *Streptomyces*, from which approximately 70% of clinically useful antibiotics are derived [206–210]. Indeed, rapamycin and ionomycin each activate AMPK and

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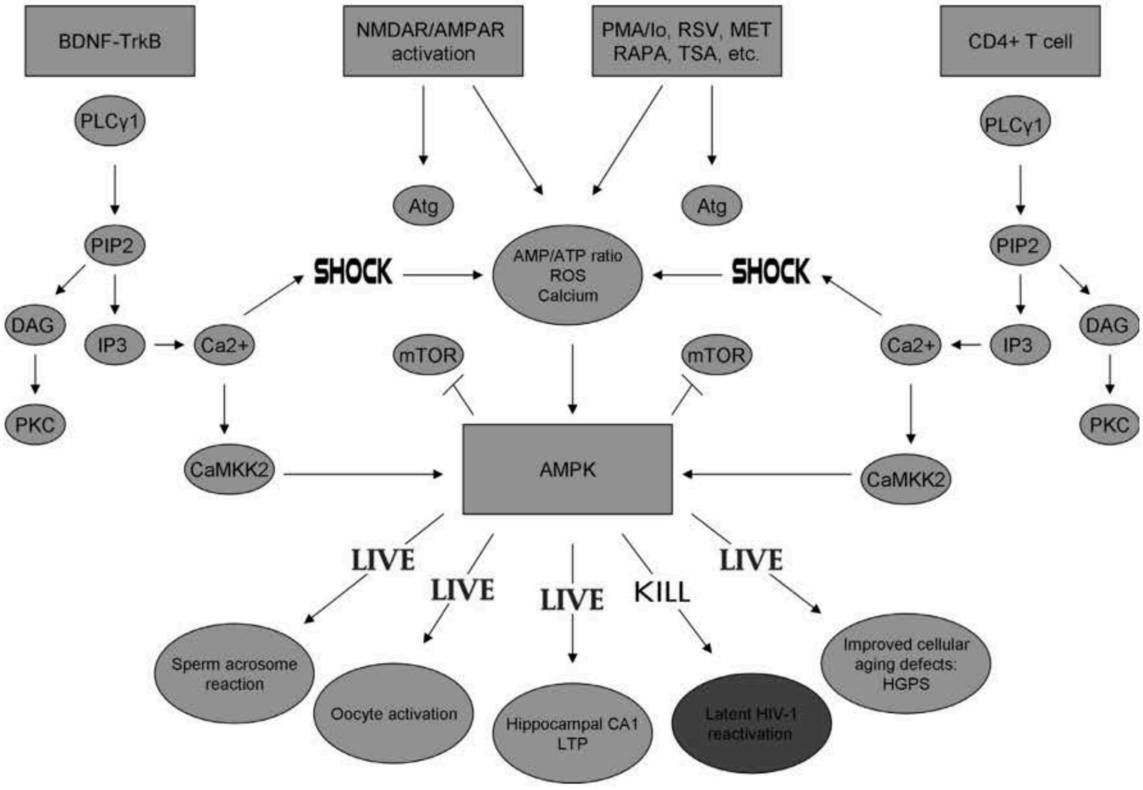

Fig. 1. Proposed mechanisms that link hippocampal CA1 LTP with latent HIV-1 reactivation, oocyte activation, sperm acrosome reaction, and HGPS. AMPK: AMP-activated protein kinase, MET: metformin, RSV: resveratrol, PMA/Io: phorbol 12-myristate 13-acetate/ionomycin, RAPA: rapamycin, TSA: trichostatin A, mTOR: mammalian target of rapamycin, AMP: adenosine monophosphate, ATP: adenosine triphosphate, ROS: reactive oxygen species, Atg: Autophagy, HIV-1: human immunodeficiency virus type 1, HGPS: Hutchinson-Gilford progeria syndrome, CaMKK2: calcium/calmodulin-dependent protein kinase kinase 2, PLCγ1: phospholipase C, gamma 1, PIP2: phosphatidylinositol 4,5-bisphosphate, DAG: diacylglycerol, PKC: protein kinase C, IP3: inositol 1,4,5-trisphosphate, Ca2+: calcium, BDNF: brain-derived neurotrophic factor, TrkB: tropomyosin receptor kinase B, NMDAR: N-methyl-D-aspartate receptor, AMPAR: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor.

are derived from various *Streptomyces* strains, with the antibiotic ionomycin often used to induce human oocyte activation during fertility procedures, giving rise to normal healthy children [29,50,181,211,212]. As beneficial levels of ROS in human follicular fluid is also associated with successful human pregnancy, cellular stress and AMPK activation likely links the creation of human life with TE activation and/or transposition, memory formation, viral eradication, and evolution of the human genome [213].

Hypothesis testing

Knockdown or pharmacological inhibition of both AMPK catalytic subunits (AMPKα1 and AMPKα2) in hippocampal neurons (e.g. hippocampal CA1 pyramidal neurons) would be conducted to determine if AMPK activation is essential for the induction, expression, and/or maintenance of LTP *in vitro* or the facilitation of learning and memory *in vivo*. Knockdown or inhibition of AMPKα1 and AMPKα2 in cell models of HIV-1 latency (e.g. T cells, macrophages, etc.) as well as in immune cells derived from HIV-1-infected patients would be conducted to determine if AMPK activation is essential for reactivation of latent HIV-1 by positive controls (e.g. PMA/Io, anti-CD3/anti-CD28 mAbs, etc.) or by candidate latency reversal agents (e.g. bryostatin-1, resveratrol, etc).

Conclusion

In conclusion, AMPK activation via the induction of cellular stress, mediated by increases in intracellular Ca^{2+} concentration, ROS generation, and/or an increase in the AMP(ADP)/ATP ratio, etc. links the reactivation of latent HIV-1 reservoirs (i.e. the "shock and kill" approach) with hippocampal CA1 LTP (i.e. the "shock and live" approach). A beneficial response (i.e. "live") to the induction of cellular stress (i.e. "shock") mediated by AMPK also links hippocampal CA1 LTP to other seemingly disparate physiological and patho-physiological processes (Fig. 1). Metformin has recently been shown to activate AMPK, beneficially alter gene splicing, and reverse accelerating aging defects in HGPS cells whereas rapamycin activates AMPK *in vivo* and reverses the cellular phenotype in HGPS. Temsirolimus, a rapamycin analog, also partially rescues the HGPS cellular phenotype but increases ROS and superoxide levels in HGPS cells within the first hour of treatment, indicting that a "shock and live" approach is also responsible for the beneficial effects of structurally dissimilar compounds in HGPS. The induction of a cellular stress response orchestrated by AMPK also links latent HIV-1 reactivation and hippocampal CA1 LTP with the creation of all human life, as ROS, PMA, and the calcium ionophore ionomycin each activate AMPK and induce the acrosome reaction in human sperm while the activated form of AMPK has been found localized in human spermatozoa with prominent staining at the acrosome. Activation of AMPK by ROS also leads to oocyte meiotic resumption (a process critical for efficient oocyte activation), PMA induces mouse oocyte activation, and ionomycin is extensively used to activate human oocytes during *in vitro* fertilization procedures, giving rise to normal healthy children, indicating that the creation of life is a contextual redefinition of the "shock and kill" approach. Very much similar to the creation of human life, the induction of hippocampal CA1 LTP and T cell activation (and thus T cell activation-induced latent HIV-1 reactivation) are dependent on intracellular Ca^{2+} increases whereas a decrease in the levels of ROS significantly inhibits T cell activation and LTP. Receptors that are critical for mediating LTP, including NMDARs,

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AMPARs, and TrkB receptors are also found on T cells and modulate T cell activation and ROS derived from mitochondria and NADPH oxidases play critical roles in LTP and T cell activation. AMPK is localized in hippocampal CA1 dendrites, is critical for T cell activation, and AMPK or CaMKK2 knockdown inhibits HIV-1 replication. CaMKK2 also promotes hippocampal CA1 LTP, AMPK activation increases the surface level expression of hippocampal AMPARs, and TCR mediated-T cell activation and BDNF-TrkB signaling are both dependent on PLCγ1. T cell activation and BDNF also activate AMPK and induce latent HIV-1 reactivation, providing compelling evidence that cellular stress-induced AMPK activation links hippocampal CA1 LTP and latent HIV-1 reactivation. Intriguingly, a recent cohort study including over 40,000 diabetic men demonstrated that metformin reduced the likelihood of cancer, dementia, depression, frailty-related diseases, and cardiovascular diseases, provocatively indicating that the induction of an AMPK-mediated cellular stress response links improvements in lifespan and healthspan with the potential eradication of HIV-1, learning and memory, and the creation of all human life [187].

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AMERICAN OVERSIGHT

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From: Sullivan, Kevin
Sent: Sun, 6 May 2018 13:45:07 +0000
To: Mark Dybul;Barbara Bush;Fauci, Anthony (NIH/NIAID) [E]
Subject: Re: UPDATED Draft Questions for Engage program
Attachments: Lazarus Effect Engage Moderator Questions v2 5 5 18 .docx

Thanks so much, Mark – attached is a clean copy for Barbara's review – and of course, Barbara – this is intended simply as a guide for you – I'm sure you will have additional questions and updates. Let me know how I can help on this end…

Will keep you posted re: Bridget Chisenga (no update) and tomorrow will send around thoughts on clips to use from Bending the Arc…and we will have the short SOTU announcement clip ready – think that could be cool to use with Gerson…

Thanks all,

Sully

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(b)(6)

From: Mark Dybul (b)(6)
Date: Saturday, May 5, 2018 at 5:10 PM
To: "Sullivan, Kevin" (b)(6)
Cc: Barbara Bush (b)(6) "Fauci, Anthony (NIH/NIAID) [E]" (b)(6)
Subject: Re: UPDATED Draft Questions for Engage program

great job pulling it together sully.

attached please find some suggestions.

happy weekend all
mark

AMERICAN OVERSIGHT

On Sat, May 5, 2018 at 7:24 AM, Sullivan, Kevin (b)(6) wrote:
Hi Barbara (and Mark and Tony)….

Attached is an updated rundown of questions for Wednesday's panel. Thanks to Mark and Tony for their guidance here. There are a few loose ends:

(b)(4)

Please send comments, questions and suggestions and on Monday I will send around an updated draft.

Thanks so much for all you are doing to make this program both memorable and inspirational…

Sully

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AMERICAN OVERSIGHT

May 5, 2018

The Lazarus Effect: 15 Years Later // Suggested Questions

Panel 1 Theme: Discuss the origin of PEPFAR: The largest international health initiative in history for a single disease which saved lives and changed the paradigm. Participants:

- Former Rwandan Minister of Health **Dr. Agnes Binagwaho**
- Key architect of PEPFAR **Ambassador Mark Dybul, M.D.**
- *Washington Post* columnist **Michael Gerson**

Suggested Questions / Framework:

[Note: Will adjust if Bridget Chisenga is added to the program with her survivor testimonial]

[Will add a few PEPFAR bulleted facts and intro bio bullets for you to consider as you begin the program]

Begin with Mark:

Q: Take us back to 2002. Can you walk us through what was happening with the AIDs epidemic in Africa…and how global leaders were attempting to address it with the Global Fund?

Q: When did you first hear that dad wanted the United States to take the lead and do something much more bold and ambitious – and what happened next?

(Note: I can take it up to the oval meeting when mike got deeply involved)

For Gerson:

Q: In November 2002 there was a pivotal meeting the Oval Office when dad told you and his senior team about the bold vision he wanted to announce in the State of the Union address. Can you describe that meeting for us and what happened next?

[BPB: We could consider playing a short clip from the 2003 SOTU here]

For Dr. Agnes:

Q: While all this was taking place in Washington, you were a pediatrician in Rwanda, working with HIV patients in clinics and public hospitals. What did the PEPFAR announcement mean to the people of Rwanda?

AMERICAN OVERSIGHT

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May 5, 2018

For Mark and Mike:

Q: Making the announcement was just the beginning. How did dad build bipartisan support for PEPFAR on the Hill and move the bureaucracy to get it done?

For Dr. Agnes:

Q: Perhaps one of the transformational, animating principles and legacies of PEPFAR was the shift from a paternalistic approach to one of true partnership. Rwandan President Paul Kagame said, "This is the first time someone respected us enough to hold us accountable." As an African medical practitioner, how did you feel about the accountability component?

For Mark:

Q: Are there other lasting legacies of PEPFAR beyond the shift from paternalism to partnership we just discussed with Agnes?

For Mike:

Q: Our next panel will talk about the state of affairs in Africa today…and the prospect of controlling, and ultimately we hope, ending the HIV epidemic. You've described PEPFAR as taking the African people from hopeless to hopeful. Let's conclude our discussion by having each of you offer a reflection on what your work in those early days means to you today…and how hopeful you are for the impact of PEPFAR to live on in sub-Saharan Africa.

Mike?

Dr. Agnes?

Mark, we'll give you the last word

AMERICAN OVERSIGHT

May 5, 2018

Panel 2: The state of health in Africa today…and looking forward, getting to the end of the epidemic. Participants:

- Key architect of PEPFAR **Dr. Anthony Fauci**
- Partner in Health's **Dr. Paul Farmer**
- **Favorite "Favo" Iradukunda**, former Global Health Corp Fellow from Rwanda
- **Senator Lindsey Graham** (R-South Carolina)

BPB: Thank you Mark, Mike and Dr. Agnes for being here…as we re-set the stage for our next panel, here's a short clip from the film, Bending the Arc…

[Play Bending the Arc "before and after" clip during the set change]

BPB: That was incredible. One of the filmmakers of Bending the Arc, **Cori Shepherd Stern**, is with us here tonight (confirm)…welcome Cori and thank you for helping tell the story of PEPFAR through this powerful film (applause)

BPB: Intro second panel (bio bullets to come)

For Tony:

Q: What is the current state of the HIV pandemic and what does the future look like?

Note - Defer to tony but seems important to link to allow for the full message to be delivered comrephensively. Would be possible to ask back to back but then the senator might get antsy

Note: This question gives Tony the chance to explain that there is a big difference between having the tools available and being able to end it…that we can't misunderstand this point in the U.S. and walk away – perfect set up for Sen. Graham)

For Sen. Graham:

Q: Senator Graham – thank you for your remarkable leadership to make sure the funding for PEFAR has continued. Before we get to funding, why is this issue so personally important to you?

Q: What are the prospects for continued U.S. leadership and funding?
(Set him up to show his funding slide)

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For Favo:

Q: From your standpoint as a nurse who has worked with patients on the ground in Rwanda, what is happening today?

Q: How do the young people of Rwanda feel about prevention…and the state of the epidemic?

For Paul:

Q: As an infectious disease doctor, you've been on the ground, working around the world in the most desperate areas. How has PEPFAR provided an infrastructure…and through the new paradigm we talked about earlier... a foundation to take on other diseases?

Q: From your perspective on the ground, what is your reaction to Dr. Fauci's comments on where we are with controlling the HIV pandemic and Favo's comments on the risk of HIV among young people?

For Favo:

Q: The effort to end the epidemic goes beyond traditional medical intervention. When you finish your Ph.D. at U Mass-Amherst, you plan to return to Rwanda and help educate nurses while also working in the community yourself. What will you tell your nursing students are the most important steps they can take - especially in terms of educating young people – to help end the epidemic?

Wrap-up question for each panelist

Q: As we've heard, there has been tremendous progress and there are many other problems in the world and here at home. From each of your perspectives, why should the American people still care?

Paul?

Favo?

Tony?

Senator Graham, we'll give you the last word…

[Remain on stage as Ken Hersh comes up for closing comments]

AMERICAN OVERSIGHT

From: Dieffenbach, Carl (NIH/NIAID) [E]
Sent: Sun, 6 May 2018 11:43:47 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: RE: Call

Tony,

You can call me now, I am doing email and looking at the paper.
(b)(6)

Carl

-----Original Message-----
From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Sunday, May 6, 2018 7:26 AM
To: Dieffenbach, Carl (NIH/NIAID) [E] (b)(6)
Subject: Call

Carl:
I am in (b)(6) to give a plenary at the ASM meeting (pandemic flu). Return this afternoon. I need to speak with you today about something. When is a good time to call?
Thanks,
Tony

AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Sat, 5 May 2018 16:20:00 +0000
To: Lane, Cliff (NIH/NIAID) [E]
Subject: RE: your trip

OK. Thanks. On another note, you have saved (b)(6) I was whining to myself because I have to go to (b)(6) late this afternoon to deliver an early morning plenary at the ASM Virology meetings tomorrow on universal influenza vaccines. (b)(6) (b)(6) Compared to what you are doing, this is like going for a walk around the block.

From: Lane, Cliff (NIH/NIAID) [E]
Sent: Saturday, May 05, 2018 12:16 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: Re: your trip

I have the abstract template and data with me and will be working on a first draft between here and (b)(6) I have discussed with Michele and Jeff - they are in agreement with sending something in to the meeting.

On May 5, 2018, at 12:00 PM, Fauci, Anthony (NIH/NIAID) [E] (b)(6) wrote:

The thought just occurred to me that since you will be away for (b)(6) the due date for the late breaker at IAS will occur while you are away. I still have not heard anything from Jeff, and you and I in addition to Jeff and Michele need to carefully craft how we word the abstract to be both honest and flexible enough for future insights, as per our discussion. How are we going to work that with you on planes and multiple time zones away for a week?

Anthony S. Fauci, MD
Director
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National Institutes of Health
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AMERICAN OVERSIGHT

From: Sullivan, Kevin
Sent: Sat, 5 May 2018 11:24:35 +0000
To: Barbara Bush;mrd54;Fauci, Anthony (NIH/NIAID) [E]
Subject: UPDATED Draft Questions for Engage program
Attachments: Lazarus Effect Engage Moderator Questions 5 4 18.docx

Hi Barbara (and Mark and Tony)….

Attached is an updated rundown of questions for Wednesday's panel. Thanks to Mark and Tony for their guidance here. There are a few loose ends:

- Monday I will add intro bio bullets for Barbara to use. (There will also be bio slides of all the participants scrolling during our pre-show in the auditorium). Will also include a few PEPFAR facts for you.

- We need to determine the best way to use Bridget Chisenga if she makes it to Dallas (stand-alone testimonial is the recommendation here…but she could be added to the first panel).

- We will play the Bending the Arc "before and after treatment" clip to cover our set change between the two panels, but on Monday I will also have a recommendation about where we could have another clip or two available for use.

Please send comments, questions and suggestions and on Monday I will send around an updated draft.

Thanks so much for all you are doing to make this program both memorable and inspirational…

Sully

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AMERICAN OVERSIGHT

May 5, 2018

The Lazarus Effect: 15 Years Later // Suggested Questions

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Q: When did you first hear that dad wanted the United States to take the lead and do something much more bold and ambitious?

For Gerson:

Q: In November 2002 there was a pivotal meeting the Oval Office when dad told you about the bold vision he wanted to announce in the State of the Union address. Can you describe that meeting for us and what happened next?

[BPB: We could consider playing a short clip from the 2003 SOTU here]

For Dr. Agnes:

Q: While all this was taking place in Washington, you were a pediatrician in Rwanda, working with HIV patients in clinics and public hospitals. What did the PEPFAR announcement mean to the people of Rwanda?

AMERICAN OVERSIGHT

May 5, 2018

For Mark and Mike:

Q: Making the announcement was just the beginning. How did dad build bipartisan support for PEPFAR and cut through the customary interagency policy process to get it done?

Q: We have Senator Lindsey Graham here tonight. Talk about the role he played to make PEPFAR happen.

For Dr. Agnes:

Q: The lasting lesson of PEPFAR is the transformation from the paternalistic approach to one of true partnership. Rwandan President Paul Kagame said, "This is the first time someone respected us enough to hold us accountable." As a medical practitioner, how did you feel about the accountability component?

For Mark:

Q: The paradigm shifted from one of services provided and money spent to results achieved and good governance…from government-to-government to people-to-people. The private sector was meaningfully engaged, especially the pharmaceutical companies. As a global health expert, what has changing this paradigm meant around the world?

For Mike:

Q: Our next panel will talk about the state of affairs in Africa today…and the prospect of eradicating the AIDS epidemic. You've described PEPFAR as taking the African people from hopeless to hopeful. Let's conclude our discussion by having each of you offer a reflection on what your work in those early days means to you today…and how hopeful you are for the impact of PEPFAR to live on in sub-Saharan Africa.

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AMERICAN OVERSIGHT

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BPB: Intro second panel (bio bullets to come)

For Tony:

Q: What is the current state of the HIV pandemic?

Q: What is the state of the science available to control the epidemic? What does the future look like?

Note: This question gives Tony the chance to explain that there is a big difference between having the tools available and being able to end it…that we can't misunderstand this point in the U.S. and walk away – perfect set up for Sen. Graham)

For Sen. Graham:

Q: Senator Graham – thank you for your remarkable leadership to make sure the funding for PEFAR has continued. Before we get to funding, why is this issue so personally important to you?

Q: What are the prospects for continued U.S. leadership and funding?
(Set him up to show his funding slide)

AMERICAN OVERSIGHT

May 5, 2018

For Favo:

Q: From your standpoint as a nurse who has worked with patients on the ground in Rwanda, what is happening today?

Q: How do the young people of Rwanda feel about prevention…and the state of the epidemic?

For Paul:

As an infectious disease doctor, you've been on the ground, working around the world in the most desperate areas. How has PEPFAR provided an infrastructure…and through the new paradigm we talked about earlier... a foundation to take on other diseases?
For Tony and Paul:

Q: Looking to the future, how close are we to an HIV vaccine? And can we eradicate the epidemic without a vaccine?

For Favo:

Q: The effort to end the epidemic goes beyond traditional medical intervention. When you finish your Ph.D. at U Mass-Amherst, you plan to return to Rwanda and help educate nurses while also working in the community yourself. What will you tell your nursing students are the most important steps they can take - especially in terms of educating young people – to help end the epidemic?

Wrap-up question for each panelist

Q: HIV/AIDS in Africa seems to be controlled. Why should the American people still care?

Paul?

Favo?

Tony?

Senator Graham, we'll give you the last word…

[Remain on stage as Ken Hersh comes up for closing comments]

AMERICAN OVERSIGHT

HHS-NIAID-20-0593-A-000998

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Fri, 4 May 2018 17:49:08 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Launch of universal flu vaccine study with Dina Maron
Attachments: RE: Interview request: Launch of universal flu vaccine study, Re: Interview with Dr. Fauci Launch of universal flu vaccine study

AMERICAN OVERSIGHT

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Fri, 4 May 2018 17:27:42 +0000
To: Stover, Kathy (NIH/NIAID) [E]
Cc: NIAID FOG;NIAID COGCORE;NIAID Media Inquiries
Subject: RE: Interview request: Launch of universal flu vaccine study

ASF will do this.

Meaghan – can you please schedule for next week. thx

Patricia L. Conrad
Special Assistant to the Director

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From: Stover, Kathy (NIH/NIAID) [E]
Sent: Friday, May 04, 2018 1:19 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: NIAID FOG (b)(6) NIAID COGCORE (b)(6) NIAID Media Inquiries (b)(6)
Subject: Interview request: Launch of universal flu vaccine study

Dina Fine Maron
Editor, Health & Medicine
Scientific American
Phone: (b)(6)
E-mail: dmaron@sciam.com
Spokesperson: Dr. Fauci
Deadline: Would like to schedule for early next week
Clearance: Not yet sought

Hi Patty,

Dina got our press release about the launch of the universal flu vaccine study (pasted below), and she'd like to talk to Dr. Fauci early next week once she's had a chance to do a little homework. In particular, she'd like to find out how BiondVax's vaccine is different from the one she wrote about here: https://www.scientificamerican.com/article/scientists-move-closer-to-a-universal-flu-vaccine/.

Would he like to speak with her?

Best,

AMERICAN OVERSIGHT

Kathy

Kathy Stover
Branch Chief, News and Science Writing Branch
National Institute of Allergy and Infectious Diseases
National Institutes of Health
31 Center Drive, Room 7A17E
Bethesda, MD 20892
Direct: (b)(6)
Media Line: (b)(6)

AMERICAN OVERSIGHT

From: Maron, Dina
Sent: Fri, 4 May 2018 17:47:06 +0000
To: Vance, Meaghan (NIH/NIAID) [C]
Subject: Re: Interview with Dr. Fauci Launch of universal flu vaccine study

Hi there,

Monday at 4:30 would work. Thanks. What number should I call?

Dina Fine Maron, MPH
Editor, Health & Medicine
Scientific American
o: (b)(6) (D.C.)
Twitter: @dina_maron
www.scientificamerican.com
Articles: http://goo.gl/kau6km

From: "Vance, Meaghan (NIH/NIAID) [C]" (b)(6)
Date: Friday, May 4, 2018 at 1:45 PM
To: "Maron, Dina" (b)(6)
Subject: Interview with Dr. Fauci Launch of universal flu vaccine study

Dina:

Dr. Fauci would be happy to speak with you. He has the following availability:

Monday, May 7th at 4:30pm EST
Tuesday, May 8th at 12:00pm or 3:00pm EST

Will any of these times work for you?

Meaghan Vance
Office of the Director
(b)(6)
(b)(6)

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AMERICAN OVERSIGHT

From: Paules, Catharine (NIH/NIAID) [E]
Sent: Fri, 4 May 2018 19:58:08 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Folkers, Greg (NIH/NIAID) [E];Conrad, Patricia (NIH/NIAID) [E];Stover, Kathy (NIH/NIAID) [E];Vance, Meaghan (NIH/NIAID) [C];Auchincloss, Hugh (NIH/NIAID) [E];Hudgings, Carole (NIH/NIAID) [E];Marston, Hilary (NIH/NIAID) [E];Eisinger, Robert (NIH/NIAID) [E];Doepel, Laurie (NIH/NIAID) [E];Billet, Courtney (NIH/NIAID) [E]
Subject: Biondvax information
Attachments: Biondvax Information 5-4-18.docx
Importance: High

Hi Dr. Fauci,
I prepared this one pager of information on the Biondvax Phase 2 trial with help from DMID. Let me know if theres anything else you need, Cat

AMERICAN OVERSIGHT

Page 0943 of 2068

Withheld pursuant to exemption

(b)(5)

of the Freedom of Information and Privacy Act

AMERICAN OVERSIGHT

Page 0944 of 2068

Withheld pursuant to exemption

(b)(5)

of the Freedom of Information and Privacy Act

AMERICAN OVERSIGHT

From: (b)(6)
Sent: Fri, 04 May 2018 15:53:45 -0400
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: RE: Sen. Bill Frist: Medicine as Currency for Peace: How Global Health Funding Could Change the World
Importance: Normal

Yeah I remembered that and had been saying they were all told day of. He probably confused with a meeting after the announcement to help settle them down.....many people adjust timing of events around the announcement as you well know.

Mark

Sent via the Samsung Galaxy S7, an AT&T 4G LTE smartphone

-------- Original message --------
From: "Fauci, Anthony (NIH/NIAID) [E]" (b)(6)
Date: 5/4/18 3:24 PM (GMT-05:00)
To: (b)(6)
Subject: RE: Sen. Bill Frist: Medicine as Currency for Peace: How Global Health Funding Could Change the World

(b)(6)

Best,

Tony

From: mrd54 (b)(6)
Sent: Thursday, May 03, 2018 7:39 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: Fwd: Sen. Bill Frist: Medicine as Currency for Peace: How Global Health Funding Could Change the World

Tony

AMERICAN OVERSIGHT

You were probably sent this as well, but a more complete description of the meeting with congressional leaders just before sotu.

Good thing they didn't all know the "top scientists" were just 2 of us. Although you count for hundreds.....

Sent via the Samsung Galaxy S7, an AT&T 4G LTE smartphone

-------- Original message --------

From: (b)(6)

Date: 5/3/18 5:44 PM (GMT-05:00)

To:

Subject: Sen. Bill Frist: Medicine as Currency for Peace: How Global Health Funding Could Change the World

Senator Frist wanted to share with you his article in Forbes published today titled "Medicine As Currency For Peace: How Global Health Funding Could Change The World." It details his personal story on how he came to view medicine as a currency for peace, starting with treating patients in a school house clinic in a remote part of Sudan, and how that experience shaped his work in Washington on the historic PEPFAR legislation. As you know, that investment today continues to be one of our most important global strategic initiatives, and something lawmakers can continue to learn from.

Best,

(b)(6)

AMERICAN OVERSIGHT

(b)(6)

AMERICAN OVERSIGHT

From: Barton Haynes, M.D.
Sent: Fri, 4 May 2018 19:39:43 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: RE: Tony Fauci for the 2019 Hideyo Noguchi Africa Prize

Ok I have a call scheduled with max on Monday. will do. best bart

---

From: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Sent: Friday, May 04, 2018 3:37 PM
To: Barton Haynes, M.D. (b)(6)
Subject: RE: Tony Fauci for the 2019 Hideyo Noguchi Africa Prize

Bart:
As per our discussion today, when you speak with Max on Monday, ask him which one between Tadamitsu Kishimoto and Tasuku Honjo would he choose to write a letter for me. Another person that we might consider if these fall through is to ask (through Tom Quinn who is a good friend of his) Nelson Sewankambo of Makerere University in Kampala, Uganda. Let us discuss again next week.
Best regards,
Tony

From: Barton Haynes, M.D. (b)(6)
Sent: Friday, May 04, 2018 7:06 AM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: FW: Tony Fauci for the 2019 Hideyo Noguchi Africa Prize

Hi Tony; see the note below from Slim.

Do you know Pontiano Kaleebu? Pioneer in the AIDS epidemic in Africa. I can ask him if you want to substitute him in for Slim. Anyone else you want me to ask?

All the best Bart

From: Salim Abdool Karim (b)(6)
Sent: Friday, May 04, 2018 3:21 AM
To: Barton Haynes, M.D. (b)(6)
Cc: Kelly Soderberg (b)(6)
Subject: RE: Tony Fauci for the 2019 Hideyo Noguchi Africa Prize

Hello, Bart.

Lovely to hear from you. It would ordinarily have been a pleasure to write a letter of support for Tony. However, I do not think that this would be appropriate as I am being nominated by the Japanese for this award. I accepted the nomination as the previous winners in this prize for research in Africa have not been African – much to my surprise. Since there is a keen interest in having an African win the prize, I accepted the nomination as an African scientist for this award. Had I known that Tony was going to be nominated, I would have happily turned down the

AMERICAN OVERSIGHT

nomination, but it is too late to do so now given the amount of work done by the Japanese scientists in dealing with my nomination. Sorry about this.
Yours
Slim
****

Professor Salim S. Abdool Karim
CAPRISA Professor of Global Health in Epidemiology: Columbia University
Director: CAPRISA – Centre for the AIDS Programme of Research in South Africa
Pro Vice-Chancellor (Research), University of KwaZulu-Natal
Director: DST-NRF Centre of Excellence in HIV Prevention
Adjunct Professor of Medicine: Cornell University
Associate: Ragon Institute of MGH, MIT and Harvard
Tel: (b)(6)
****

From: Barton Haynes, M.D. (b)(6)
Sent: Friday, 04 May 2018 3:39 AM
To: Salim Abdool Karim (b)(6)
Cc: Kelly Soderberg (b)(6)
Subject: Tony Fauci for the 2019 Hideyo Noguchi Africa Prize

Hi Salim:

I hope you are well.

This year I am nominating Tony Fauci for the 2019 Hideyo Noguchi Africa Prize that is given once every 5 years for Medical Research For Africa. You know Tony so well, and I would be grateful if you would consider writing one of the supporting letters for this award for Tony.

If you will write a supporting letter, for your ease in doing so, I have attached the nomination of Tony so you can see his nomination and listing of accomplishments.

If you can write for Tony, it would be great if we can receive the letter by June 15th so we can put the nomination together and submit.

Many thanks for considering this support letter for Tony.

My very best to you,

Bart

Barton F. Haynes, M.D.
Director, Duke Human Vaccine Institute
Frederic M. Hanes Professor of Medicine and Immunology
2 Genome Court
MSRBII Bldg. Room 4090

AMERICAN OVERSIGHT

DUMC 103020
Duke University Medical Center
Durham, NC 27710
Ph: (b)(6)
Fx: 919-684-5230
(b)(6)

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Fri, 4 May 2018 19:36:38 +0000
To: Barton Haynes, M.D.
Subject: RE: Tony Fauci for the 2019 Hideyo Noguchi Africa Prize

Bart:
As per our discussion today, when you speak with Max on Monday, ask him which one between Tadamitsu Kishimoto and Tasuku Honjo would he choose to write a letter for me. Another person that we might consider if these fall through is to ask (through Tom Quinn who is a good friend of his) Nelson Sewankambo of Makerere University in Kampala, Uganda. Let us discuss again next week.
Best regards,
Tony

From: Barton Haynes, M.D. (b)(6)
Sent: Friday, May 04, 2018 7:06 AM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: FW: Tony Fauci for the 2019 Hideyo Noguchi Africa Prize

Hi Tony; see the note below from Slim.

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Subject: RE: Tony Fauci for the 2019 Hideyo Noguchi Africa Prize

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Yours
Slim
****

Professor Salim S. Abdool Karim
CAPRISA Professor of Global Health in Epidemiology: Columbia University

AMERICAN OVERSIGHT

Director: CAPRISA – Centre for the AIDS Programme of Research in South Africa
Pro Vice-Chancellor (Research), University of KwaZulu-Natal
Director: DST-NRF Centre of Excellence in HIV Prevention
Adjunct Professor of Medicine: Cornell University
Associate: Ragon Institute of MGH, MIT and Harvard
Tel: (b)(6)
****

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Sent: Friday, 04 May 2018 3:39 AM
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Cc: Kelly Soderberg (b)(6)
Subject: Tony Fauci for the 2019 Hideyo Noguchi Africa Prize

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Many thanks for considering this support letter for Tony.

My very best to you,

Bart

Barton F. Haynes, M.D.
Director, Duke Human Vaccine Institute
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2 Genome Court
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From: Paules, Catharine (NIH/NIAID) [E]
Sent: Fri, 4 May 2018 17:57:06 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: FW: Quick turnaround request for Dr. Fauci
Importance: High

Chris Robert is working on this now. Will have an answer for you shortly. Thanks, cat

---

From: Paules, Catharine (NIH/NIAID) [E]
Sent: Friday, May 04, 2018 1:41 PM
To: NIAID BUGS (b)(6)
Cc: Glowinski, Irene (NIH/NIAID) [E] (b)(6) Roberts, Chris (NIH/NIAID) [E] (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: Quick turnaround request for Dr. Fauci
Importance: High

Hi BUGS,
I have a quick turnaround request for Dr. Fauci which he needs in the next 1-2 hours for a media inquiry about the NIAID-sponsored universal flu vaccine using the Biondvax product. He would like a short (1-2) sentence scientific rationale for why we are choosing to prime with the Biondvax product and then boost with the seasonal vaccine. I have some very helpful information on the product that Chris Roberts sent me previously so we just need a brief statement on the scientific rationale. Happy to get on the phone if helpful, Cat

AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Fri, 4 May 2018 13:19:47 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Press Club Universal flu event (Bill Pierce)
Attachments: FW: connecting to Dr. Fauci's office for March 29 briefing, RE: New dates needed, FW: NIAID Director Dr. Anthony Fauci to discuss progress made in the development of a universal flu vaccine at National Press Club Headliners Luncheon July

they are from 12-2 pm generally and include a 30 minutes VIP reception beforehand, a luncheon (30 minutes to eat; an hour of remarks that includes a moderated Q&A with audience; those in-person write questions on cards that are passed up to the head table where someone sorts and puts to the moderator to ask, plus ones come in via email and twitter.).

CONTACT: Lindsey Underwood cell cell is: (b)(6)

REVISED: Lunch will be served in the club's Holeman Lounge at 12:30 p.m., with remarks beginning at 1 p.m., followed by a question-and-answer session ending at 2 p.m. Tickets cost $25 for National Press Club members (members may purchase two tickets at this rate) and $39 for all other non-member tickets.

RUN-OF-SHOW:

12:00 PM: Head Table Reception in Bloomberg Room.

12:25 PM: Head Table seated in the Holeman Lounge,
The moderator and the speaker stay behind. The moderator and speaker have a few photos taken together by the club's official photographer, and then the speaker signs the guest book and autographs his or her headshot that will later hang in the gallery outside of the ballroom.

12:30 PM: The speaker and moderator enter the Holeman Lounge and are seated at the Head Table for lunch.

1:00 PM: The moderator goes up to the podium and delivers brief welcoming remarks and an introduction of the speaker.

1:05 – 1:25/1:30 PM: Speaker delivers remarks from the podium (usually 20-25 minutes).

1:25/1:30 PM: Moderator joins speaker at podium to begin Q&A portion of the event (peppering in audience questions that are submitted throughout the program on question cards).

1:55 PM: Moderator announces upcoming headliner events, asks 1 more "kicker" question of the speaker, and presents the speaker with an official NPC Mug.

2:00 PM: Program ends.

AMERICAN OVERSIGHT

AMERICAN
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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Fri, 4 May 2018 13:18:27 +0000
To: Conrad, Patricia (NIH/NIAID) [E]
Subject: FW: connecting to Dr. Fauci's office for March 29 briefing

Patricia L. Conrad
Special Assistant to the Director

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From: Pierce, William (b)(6)
Sent: Friday, April 06, 2018 4:15 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: RE: connecting to Dr. Fauci's office for March 29 briefing

I am back. Wanted to follow-up per my ask re Dr. Fauci doing a National Press Club Headliners luncheon (this is our premier speaking venue) similar to what he did at the Brazda Breakfast except this would be before an audience of 200 plus, C-Span guaranteed and possibly other tv and radio, plus print, etc. He's done many of these before but they are from 12-2 pm generally and include a 30 minutes VIP reception beforehand, a luncheon (30 minutes to eat; an hour of remarks that includes a moderated Q&A with audience; those in-person write questions on cards that are passed up to the head table where someone sorts and puts to the moderator to ask, plus ones come in via email and twitter.). He would get up to 3 guests at the head table.

If you could give me some dates when he could do say in May and June that would be great and then we can run them up against events already scheduled.

Any thoughts on when and where we should try and pull together folks for a drink?

Thanks.

Bill

William A. Pierce
Senior Director, Health Policy + Public Affairs
Lead - Media Relations, Washington Office
APCO Worldwide
1299 Pennsylvania Avenue, NW
Suite 300, Entrance 12th & E St.
Washington, D.C. 20004

AMERICAN OVERSIGHT

202-659-7931
202-352-6659 (c)

(b)(6)
https://www.linkedin.com/in/bill-pierce
www.apcoworldwide.com
@WilliamAPierce

::::: **Public Affairs Agency of the Year, 2017** - *The Holmes Report* :::::

William Pierce
APCO Worldwide
(t) (b)(6) (m) (b)(6)

From: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Sent: Thursday, March 29, 2018 12:39 PM
To: Pierce, William (b)(6)
Subject: RE: connecting to Dr. Fauci's office for March 29 briefing

Excellent – safe travels.

Patricia L. Conrad
Special Assistant to the Director

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From: Pierce, William (b)(6)
Sent: Thursday, March 29, 2018 12:37 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: Re: connecting to Dr. Fauci's office for March 29 briefing

It did as always as he is a great draw. c-Span and NBC.

When I get back I'll give you a call for drinks.

Also National Press Club is interested In him doing a Headliners luncheon address. Similar topic. I can share some dates when I get back.

Bill

Sent from my iPhone

AMERICAN OVERSIGHT

William Pierce
APCO Worldwide
(t) (b)(6) (m) (b)(6)

On Mar 29, 2018, at 5:20 PM, Conrad, Patricia (NIH/NIAID) [E] (b)(6) wrote:

Heard the event went well…..I did not attend with him – other staff here did.

Hope you are well…..keep in touch….we need to do drinks more often…I am always looking for a reason to go downtown!!! ☺

Patricia L. Conrad
Special Assistant to the Director

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From: Pierce, William (b)(6)
Sent: Friday, March 16, 2018 12:40 PM
To: Jill O'Brien (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Kathryn Martucci (b)(6) Manchester, Brittney (b)(6) Sarah Dash (b)(6) Alvarez, Susie (b)(6) Ana McKessy (b)(6)
Subject: connecting to Dr. Fauci's office for March 29 briefing

Jill meet Patty, Patty meet Jill.

Jill, I mentioned to Patty in an email that we are the partner to the Alliance in the press briefings, and that you handle most of the prep for the briefings so I wanted to introduce you so you could coordinate Dr. Fauci's visit.

I have already let Patty know we can accommodate a Power Point presentation.

Thanks.

Bill

AMERICAN OVERSIGHT

William A. Pierce
Senior Director, Health Policy + Public Affairs
Lead - Media Relations, Washington Office
APCO Worldwide
1299 Pennsylvania Avenue, NW
Suite 300, Entrance 12th & E St.
Washington, D.C. 20004
(b)(6)
(b)(6)

wpierce@apcoworldwide.com
https://www.linkedin.com/in/bill-pierce
www.apcoworldwide.com
@WilliamAPierce

William Pierce
Senior Director

APCO Worldwide
1299 Pennsylvania Ave, N.W.
Suite 300
Washington, DC 20004

(t) (b)(6) (m) (b)(6)

wpierce@apcoworldwide.com
@apcoworldwide

::::: Public Affairs Agency of the Year, 2017 - *The Holmes Report* :::::

AMERICAN OVERSIGHT

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Fri, 4 May 2018 13:19:38 +0000
To: Pierce, William
Subject: RE: New dates needed

Yes -we can make July 18 work.

Patricia L. Conrad
Special Assistant to the Director

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From: Pierce, William (b)(6)
Sent: Thursday, May 03, 2018 6:34 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: Re: New dates needed

What about July 18?

Seems like the universal flu vaccine to prepare for a pandemic is a rising topic with Gates and Trump joining the conversation.

Sent from my iPhone

William Pierce
APCO Worldwide
(t) (b)(6) (m) (b)(6)

On Apr 25, 2018, at 12:40 PM, Conrad, Patricia (NIH/NIAID) [E] (b)(6) wrote:

July 10 and 11 could now have conflict

Patricia L. Conrad
Special Assistant to the Director

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Friday, April 20, 2018 9:31 AM
To: 'Pierce, William' (b)(6)
Subject: RE: New dates needed

How about the below dates:

June 29
July 10, 11, 13, 17, 18, 19

As for drinks – Am good any night next week except the 23rd or any night the week after.....

Patricia L. Conrad
Special Assistant to the Director

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From: Pierce, William (b)(6)
Sent: Thursday, April 19, 2018 9:40 AM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: RE: New dates needed

Unfortunately no.

Other dates outside those previously provided?

William Pierce
APCO Worldwide
(t) (b)(6) (m) (b)(6)

From: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, April 18, 2018 12:30 PM

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To: Pierce, William (b)(6)
Subject: RE: New dates needed

We had Friday June 22 open up – would that work?

Patricia L. Conrad
Special Assistant to the Director

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From: Pierce, William (b)(6)
Sent: Sunday, April 15, 2018 10:22 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: New dates needed

Amazingly, none of the dates work for NPC can you, look even further forward into the calander?

Thanks.

Bill

William A. Pierce
Senior Director, Health Policy + Public Affairs
Lead - Media Relations, Washington Office
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::::: **Public Affairs Agency of the Year, 2017** - *The Holmes Report* :::::

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::::: Public Affairs Agency of the Year, 2017 - *The Holmes Report* :::::

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Thu, 14 Jun 2018 21:04:54 +0000
To: Vance, Meaghan (NIH/NIAID) [C]
Subject: FW: NIAID Director Dr. Anthony Fauci to discuss progress made in the development of a universal flu vaccine at National Press Club Headliners Luncheon July

For the July 18th folder

Patricia L. Conrad
Public Health Analyst and
Special Assistant to the Director
National Institute of Allergy and Infectious Diseases
The National Institutes of Health
31 Center Drive, MSC 2520 - Room 7A03
Bethesda, Maryland 20892
(b)(6)
301-496-4409 fax

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From: Folkers, Greg (NIH/NIAID) [E]
Sent: Thursday, June 14, 2018 5:00 PM
Subject: NIAID Director Dr. Anthony Fauci to discuss progress made in the development of a universal flu vaccine at National Press Club Headliners Luncheon July

NIAID Director Dr. Anthony Fauci to discuss progress made in the development of a universal flu vaccine at National Press Club Headliners Luncheon July 18

News provided by
National Press Club
15:26 ET

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WASHINGTON, June 14, 2018 /PRNewswire-USNewswire/ -- This past year's flu season killed more children than any other non-pandemic year on record, according to data released by the Centers for Disease Control and Prevention (CDC) earlier this year. As the world marks the 100-year anniversary of the Spanish flu pandemic, which killed an estimated 50 million people worldwide in 1918, the push for the development of a universal flu vaccine capable of addressing future pandemics is gaining ground – most notably with the appropriations of an additional $40 million to the National Institute of Allergy and Infectious Diseases (NIAID) budget for a total of $100 million to develop such a vaccine, and the Bill & Melinda Gates Foundation's announcement this past spring to award $12 million to the cause.

Leading the research on the development of a universal flu vaccine is Dr. Anthony Fauci, Director of the NIAID. With the start of the 2018-2019 flu season just a few months away, Dr. Fauci will speak about the progress made thus far, and the challenges that lay ahead as each strain grows deadlier and public trust in vaccinations wane at a National Press Club Headliners Luncheon on Wednesday, July 18.

Dr. Fauci began his career at the National Institutes of Health (NIH) back in 1968 in the Laboratory of Clinical Investigation in NIAID, and was later appointed Chief of the Laboratory of Immunoregulation in 1980, a position he still holds to this day. Dr. Fauci was appointed Director of NIAID in 1984 and has advised five Presidents on HIV/AIDS and many other domestic and global health issues. Fauci was awarded the Presidential Medal of Freedom by President George W. Bush for his role as an architect of the President's Emergency Plan for AIDS Relief (PEPFAR), which has been responsible for saving millions of lives in sub-Saharan Africa. NIAID and Dr. Fauci have been front and center on many headline-making incidents including the anthrax attacks in 2001 and the most recent Ebola outbreak that included treating several persons who treated Ebola victims in Africa.

Lunch will be served in the club's Holeman Lounge at 12:30 p.m., with remarks beginning at 1 p.m., followed by a question-and-answer session ending at 2 p.m. Tickets cost $25 for National Press Club members (members may purchase two tickets at this rate) and $39 for all other non-member tickets. Please click here to purchase tickets to the luncheon.

For all ticketing-related questions, please email reservations@press.org. Tickets must be paid for at the time of purchase. To submit a question for the speaker in advance, put FAUCI in the subject line and email to president@press.org. The deadline for submitting questions in advance is 10 a.m. on the day of the luncheon.

The National Press Club is located on the 13th Floor of the National Press Building at 529 14th St., NW, Washington, D.C.

PRESS CONTACT:

Lindsay Underwood,

(b)(6)

(b)(6)

SOURCE National Press Club

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https://www.press.org

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From: McNeil, Donald
Sent: Fri, 4 May 2018 13:11:50 -0400
To: Stover, Kathy (NIH/NIAID) [E];Fauci, Anthony (NIH/NIAID) [E]
Subject: Re: NIAID Launches Phase 2 Universal Influenza Vaccine Study

Thank you.

The start of a phase II trial seems a little early to write -- I'm always reluctant to write stories that will get headlines like "Is the End of the Flu at Hand?" because people will think the problem is solved and stop taking precautions..

But I'm confused about something.

Why is the second stage of the trial an injection with seasonal flu vaccine?

I thought this might be a challenge trial...

Or is that what it sort of is? Is this the live-virus attenuated vaccine? Is it functioning as a substitute for giving the volunteers an actual dose of flu?

Donald

On Fri, May 4, 2018 at 11:49 AM, Stover, Kathy (NIH/NIAID) [E] (b)(6) wrote:

Hi Mr. McNeil,

Hope you're having a great Friday. Just wanted to flag our release (pasted below) as I know you've covered the topic of universal influenza vaccines in the past. If you're interested in speaking with Dr. Fauci about this latest study, I'd be happy to facilitate. Just let me know.

Best,

Kathy

Kathy Stover

AMERICAN OVERSIGHT

Branch Chief, News and Science Writing Branch

National Institute of Allergy and Infectious Diseases

National Institutes of Health

31 Center Drive, Room 7A17E

Bethesda, MD 20892

Direct: (b)(6)

Media Line: (b)(6)

FOR IMMEDIATE RELEASE
Friday, May 4, 2018

Media Contact:
Elizabeth Deatrick
(b)(6)
(b)(6)

NIAID-Sponsored Trial of a Universal Influenza Vaccine Begins

A Phase 2 clinical trial of an investigational universal influenza vaccine intended to protect against multiple strains of the virus has begun in the United States. The study is sponsored by the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health, and is being conducted at four U.S. sites that are part of the NIAID-funded Vaccine and Treatment Evaluation Units (VTEUs). The trial is testing an experimental vaccine called M-001 for safety and its ability to produce potentially broad protective immune responses, both on its own and when followed by a standard, licensed seasonal influenza vaccine.

Influenza viruses mutate constantly, resulting in the emergence of viruses that may not always match those targeted by seasonal and pre-pandemic influenza vaccines. Seasonal influenza vaccines are made anew each year to match the strains predicted to circulate in the upcoming season. To receive the best protection against influenza, people must be vaccinated annually. However, if a particular influenza strain changes in an unanticipated way, or a different strain from that included in the vaccine spreads widely, the seasonal influenza vaccine may not be sufficiently protective. Each year, seasonal influenza sickens millions in the United States and results in 140,000 to 710,000 hospitalizations and between 12,000 and 56,000 deaths, according to the Centers for Disease Control and Prevention.

Influenza pandemics occur when a novel influenza strain for which people have little to no protection begins to spread among humans and present a greater public health threat than seasonal influenza.

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For example, the 1918 influenza pandemic killed at least 50 million people worldwide. An ideal universal influenza vaccine would provide durable protection for all age groups against multiple influenza strains, including those that might cause a pandemic.

"The 2017-2018 influenza season in the United States was among the worst of the last decade and serves as a reminder of the urgent need for a more effective and broadly protective influenza vaccine," said NIAID Director Anthony S. Fauci, M.D. "An effective universal influenza vaccine would lessen the public health burden of influenza, alleviate suffering and save lives. There are numerous paths of inquiry that the scientific community is pursuing, with each new study yielding more critical information and bringing us closer to our shared goal."

The new trial is being led by principal investigator Robert L. Atmar, M.D., of Baylor College of Medicine in Houston. The trial will test the M-001 vaccine candidate, developed and produced by BiondVax Pharmaceuticals based in Ness Ziona, Israel. The experimental M-001 vaccine contains antigenic peptide sequences shared among many different influenza viruses. Theoretically, it could protect against many current and emerging strains of influenza. Six previous clinical trials involving a total of 698 participants conducted by BiondVax in Israel and Europe indicated that the vaccine candidate was safe, well-tolerated and produced an immune response to a broad range of influenza strains.

The new study will enroll up to 120 healthy volunteers between the ages of 18 and 49 years. Participants will be assigned randomly to receive either two doses of the experimental vaccine or a placebo. They will be vaccinated twice, receiving one dose (1 mg; 0.4 milliliters) of M-001 or placebo via intramuscular injection on the first day and a second dose 22 days later. Approximately 172 days later, all participants will receive an approved seasonal influenza vaccine. During periodic additional clinic visits throughout the course of the study, blood will be drawn from study volunteers to evaluate their immune responses to both the experimental vaccine and to the seasonal vaccine. Each participant will be followed for approximately seven months.

The trial will take place at four NIAID-funded VTEUs. Patients will be enrolled at the Baylor College of Medicine; the University of Iowa in Iowa City; and Cincinnati Children's Hospital Medical Center. Laboratory support will be provided by Saint Louis University. For more information about the study, go to ClinicalTrials.gov, using the identifier NCT03058692.

NIAID conducts and supports research—at NIH, throughout the United States, and worldwide—to study the causes of infectious and immune-mediated diseases, and to develop better means of preventing, diagnosing and treating these illnesses. News releases, fact sheets and other NIAID-related materials are available on the NIAID website.

About the National Institutes of Health (NIH): NIH, the nation's medical research agency, includes 27 Institutes and Centers and is a component of the U.S. Department of Health and Human Services. NIH is the primary federal agency conducting and supporting basic, clinical, and translational medical research, and is investigating the causes, treatments, and cures for both common and rare diseases. For more information about NIH and its programs, visit www.nih.gov.

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National Institute of Allergy and Infectious Diseases (NIAID)
http://www.niaid.nih.gov

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--

Donald G. McNeil Jr.
Science Correspondent
The New York Times

Tel: (b)(6)
mcneil@nytimes.com

Articles: http://topics.nytimes.com/top/reference/timestopics/people/m/donald_g_jr_mcneil/index.html

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From: Kralievits, Katherine
Sent: Fri, 4 May 2018 15:59:00 +0000
To: Fauci, Anthony (NIH/NIAID) (b)(6)
Cc: Paul Farmer
Subject: The Lazarus Effect

Dear Dr. Fauci, dear Dr. Dybul,

I hope you both are well. (Dr. Fauci, it was very nice to see you at the NAS ceremony in D.C.) Paul is looking forward to seeing you both in (b)(6) this coming week. When he saw the title of the event—"The Lazarus Effect"—he was touched to remember his early Haitian patients. Yesterday, we looked up the NPR piece on it, and he asked me to send along the two links:

https://www.npr.org/programs/specials/aids/haiti.html

https://www.npr.org/2012/07/03/156154794/treating-hiv-from-impossible-to-halfway-there

Paul is looking forward to connecting on the call in a couple hours.

Sincerely,
Katie
--
Katherine Kralievits, MS
Office of Dr. Paul Farmer
Department of Global Health and Social Medicine
Harvard Medical School
(b)(6)

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Fri, 4 May 2018 13:11:17 +0000
To: Fauci, Anthony (NIH/NIAID) [E];Haskins, Melinda (NIH/NIAID) [E];Selgrade, Sara (NIH/NIAID) [E];Crawford, Chase (NIH/NIAID) [E]
Subject: discuss the May 23 Senate NIH Caucus briefing

Hi Patty,

For next week – could we meet with him to discuss the May 23 Senate NIH Caucus briefing? The topic of his talk is universal influenza vaccines.

It would be 30 minutes or less. Please let us know what might be convenient.

Thanks again,
Sara

Sara Selgrade, Ph.D.
Section Chief for Legislative Activities
Legislative Affairs and Correspondence Management Branch
Office of Communications and Government Relations
NIAID/NIH/DHHS
Bldg. 31, Room 7A17, MSC 2520
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From: mrd54
Sent: Thu, 03 May 2018 19:38:32 -0400
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Fwd: Sen. Bill Frist: Medicine as Currency for Peace: How Global Health Funding Could Change the World
Attachments: Forbes - Medicine as Currency For Peace.5.3.18.pdf
Importance: Normal

Tony

You were probably sent this as well, but a more complete description of the meeting with congressional leaders just before sotu.

Good thing they didn't all know the "top scientists" were just 2 of us. Although you count for hundreds.....

Sent via the Samsung Galaxy S7, an AT&T 4G LTE smartphone

-------- Original message --------
From: Erin Rogus (b)(6)
Date: 5/3/18 5:44 PM (GMT-05:00)
To:
Subject: Sen. Bill Frist: Medicine as Currency for Peace: How Global Health Funding Could Change the World

Senator Frist wanted to share with you his article in Forbes published today titled "Medicine As Currency For Peace: How Global Health Funding Could Change The World." It details his personal story on how he came to view medicine as a currency for peace, starting with treating patients in a school house clinic in a remote part of Sudan, and how that experience shaped his work in Washington on the historic PEPFAR legislation. As you know, that investment today continues to be one of our most important global strategic initiatives, and something lawmakers can continue to learn from.

Best,

Erin

Erin Ingraham Rogus

Senior Policy Advisor

AMERICAN OVERSIGHT

Office of Senator William H. Frist, M.D.

2525 West End Avenue, Suite 1250

Nashville, TN 37203

Cell: (b)(6)

(b)(6)

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Forbes

Medicine As Currency For Peace: How Global Health Funding Could Change The World

By Bill Frist, MD
May 3, 2018

It's 1997. We are a team of three doctors, tightly packed into a tiny, twin-engine plane, loaded to the brim with bandages, medicines and surgical supplies. We purposely lose our passports, and all personal identification, back in Uganda. Flying the last 200 miles at treetop level to avoid being spotted by circling bombers and gunships.

We're on a secret mission into a terrorist country that's fighting a bloody civil war, the Islamic Republic of Sudan. We drop fast, land in a dirt field, and then anxiously unload our supplies. We get the plane airborne and out of sight within minutes.

The next day, we set up our surgical facility in an abandoned, two-room school house. The surrounding village is completely deserted because of the ongoing, decades-long civil war. Several hundred yards away, a once thriving village hospital lies empty, ringed with active land mines. It is our haunting, daily reminder of the hate and destruction of war.

With chalk-boards pushed to the corner, we emergently operate on a badly wounded fighter, a lifesaving above-the-knee amputation. We don't know if he fights for the good guys or the bad guys – and it doesn't matter. We are there to heal. Next is a feverish, frightened young boy with a ruptured appendix. He will die without surgery. And the cases, keep coming, and coming faster, and then coming even faster.

Over the next two weeks, we operate all day, every day, and at night by flashlight.

And the most amazing thing, and the most unexpected to me--the thing that blew me away--is that the fighting around the clinic schoolhouse begins to slow. Then stops! And within weeks that newfound peace miraculously spreads throughout the entire province.

From healing grow dignity and self-worth. From that healing, grow trust and hope. Our healing presence introduces a calmness, an air of tolerance and mutual understanding. Our healing presence, introduces a currency for peace, medicine as a currency for peace.

My experience in Sudan didn't just come out of the blue. You see, I had the good luck to grow up in a medical family – dad, a family doctor, and two older brothers, physicians as well. My earliest and fondest memories as a little boy are sitting next to Dad on the

AMERICAN OVERSIGHT

front seat of his old, green Chevrolet, my left arm propped up on his well-worn, black doctor's bag.

Thousands of Sudanese attend a pro-government rally in the capital Khartoum Tuesday Jan. 14, 1997. During the address Sudanese President Omar el-Bashir ruled out any compromise with rebels of John Garang's Sudan People's Liberation Army who seized the southern Sudanese towns of Kurmuk and Qasan near the Ethiopian border this weekend. (AP Photo/Raouf Moh)

We are going on a house call.

I hold his hand tightly as we enter a darkened bedroom. From across the room, I watch with wonder as Dad slowly approaches a grey-haired woman, sits on the side of the bed, and gently touches her hand, connecting so intimately, so intensely, listening and nodding.

Time slows, He leans over to his bag, pulls out a dark yellow vial, and reassuringly gives it to the woman. Her face turns, she says something. And then she smiles. The room instantly brightens. The heavy veil of despair and depression lifts. You feel the compassion, you feel the restored hope.

So, in that room, on that day, my own lifelong journey toward health, hope and healing began. It had a natural course, leading me on to medical school, and then into surgery, and then to join pioneering surgeons exploring a radical, revolutionary new field of medicine.

You see, in the early years of my training, we had absolutely no treatment, for the otherwise healthy person who had a badly failing heart. None at all. The whole idea of saving a life, by cutting out a diseased heart and replacing it with a healthy one, was science fiction, an impossible dream. But these courageous surgeons set the goal, committed the resources, and made heart transplantation a reality. They solved what for ages had been unsolvable — they gave new life where there was no hope.

Transplanting hearts and lungs became my daily routine.

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At about the same time, little did I know that an even greater threat, a more existential threat to humanity, was just beginning to emerge. It, too, was a disease for which there was no treatment, and no cure. Its cause was a single virus, a cagey, little virus that would constantly change its shape to escape detection and treatment. The virus was HIV. The disease, AIDS.

I first met the virus as a young surgical resident in training. I read the initial 1981 report of five people in California who died of a mysterious, unnamed disease. The virus outsmarted and outran us. The first year, we watched helplessly as a few hundred people died. The next year, a few thousand, then a hundred thousand, then a million. And eventually three million people dying every year, that's more people than died in the entire Korean and Vietnam Wars combined, every year.

I realized the scale of human suffering on my annual medical mission trips to Africa. My clinics overflowed with AIDs patients. The virus hollowed out entire societies, taking first the most productive members at the prime of their lives — teachers, police, civil servants, mothers. In Botswana, life expectancy plummeted to 39 years of age!

Yes, it was time to act. Not just to listen, not just to talk, but to act.

In 1994, I ran for the US Senate, and won. As the only doctor in the Senate, I shared my medical experiences in Africa with my Senate colleagues, so they'd understand the magnitude of destruction caused by this single virus. I brought back pictures of emaciated patients lying three to a single cot, dying of AIDS.

One afternoon in my Senate Majority Leader office, I receive a call from the Office of the President of the United States. "Could you come to a small, confidential dinner with the President in the Red Room of the White House? Please say nothing to anyone about this meeting."

I know something special is up.

The next evening, I walk into the Red room and join the President and a handful of others. Over the next two hours, the President spells out in detail his ambitious, tightly held plan: The President will commit US leadership and unprecedented resources on behalf of the American people. The plan is to eradicate AIDS in Africa.

In the room someone says, "It can't be done." the President replies, "My smartest scientists tell me it can."

Another one says, "It's too expensive." The President replies, "My researchers say the cost of treatment will fall to a tenth of what it is today, if we totally commit our creativity, our innovation, and our science to it."

"It's overseas, and we've got problems right here at home." The President responds, "A person should not be condemned to die because of where they're born. It is the moral and right thing to do."

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And finally: "But Mr. President, it's never been done before." And President George W. Bush stands up: "And that's why the American people will do it."

JOHANNESBURG, SOUTH AFRICA - JANUARY 27: (SOUTH AFRICA OUT) Patients attend the Right To Care AIDS clinic on January 27, 2012 in Johannesburg, South Africa. The Right to Care non-governmental organisation has, with US funding from PEPFAR managed to revive their Alexandra based AIDS clinic. The clinic is now providing quality medical treatment to more than 8000 patients. (Photo by Foto24/Gallo Images/Getty Images)

The idea is so big and so bold! I realize based in on my own experiences, the plan, if successful, will change the course of human history. Overwhelmed, I just listen quietly. But in my head are swirling the images and memories of: Sudan's medicine as a currency for peace, transplant surgeons conquering the unconquerable, dad's bedside compassion.

Three weeks later at the Capitol, the President announced his plan at the State of the Union address. I took the proposal back to the Senate, we wrote the legislation, and with bipartisan support passed the President's Emergency Plan for Aids Relief, or PEPFAR. PEPFAR provided an astounding $15 billion to fight AIDS across Africa and the developing world — more than any country or any President has ever committed to fight a single disease. US industry stepped up. Our remarkable scientists got to work, and together, they developed powerful, life-saving, anti-retroviral drugs. In Africa, infected teachers and workers regained health to build stronger and more secure communities. In Botswana life expectancy jumped from 39 years to 63.

Life replaced death. Hope replaced despair. And stability replaced chaos.

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So, looking back today, did PEPFAR work? Twenty million people are alive today who would otherwise have died. Tens of millions more now have better access to improved healthcare for other infectious diseases, chronic illness, and maternal and child health.

But we can't rest. Regrettably, some in Washington are calling to cut our investment in global health. I only wish we could share with them what we know. The incredible success of PEPFAR; The small amount we actually spend on global health – only one quarter of 1% of our nation's budget; the reality that deadly infectious agents like Ebola and Zika are just a short plane ride away. I only wish we could share with them how medicine abroad contributes to security here at home.

History tells us that strength is the road to peace, and this is so. But there are many kinds of strength. There is the strength of arms, and there is the strength of healing hands. There is the strength of courage, and there is the strength of caring. Above all, there is the military strength that defends the life of a nation, and there is the medical strength that saves one life at a time.

Generations of Americans have learned the painful lesson of "peace through strength." And so, when it comes to military might, our nation is blessed with "the greatest strength the world has ever seen."

Now let's match that force of arms with the greatest strength the world has ever seen when it comes to medical mercy. Let's practice the lesson of "peace through healing." Let's remember that rogue regimes and hateful fanatics are not the only threats to global peace.

Disease is a threat to peace. Pandemics are a threat to peace. Illness and hopelessness are threats to peace. And so, in a world facing all of these threats, now is precisely the wrong time to cut back on our modest funding for global health.

The world is a safer and more secure place for us all when societies overseas are healthier and more stable. The world is safer and more secure for everyone when individuals provide for their families and create opportunities for their children, without the worry of being struck down by an illness that could be easily prevented or easily cured.

To be blunt, you don't go to war with someone who has just saved the life of your child.

Martin Luther King Jr. said, "Injustice anywhere is a threat to justice everywhere." In our time, we've learned that a threat to health anywhere is a threat to peace everywhere. We can bring more peace to the world and to ourselves, not only by deterrence – but also by compassion, by the power of healing hands, and by medicine as a currency for peace.

Last year, I gave a TEDMED talk outlining how medicine can serve as a currency for peace. This is my script for that talk. You can watch the video here: https://www.tedmed.com/talks/show?id=687176

AMERICAN OVERSIGHT

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Thu, 3 May 2018 20:41:31 +0000
To: Vance, Meaghan (NIH/NIAID) [C];Folkers, Greg (NIH/NIAID) [E];Fauci, Anthony (NIH/NIAID) [E]
Subject: FW: on behalf of Dr. Fauci - Please read
Attachments: Lazarus Effect Engage Outline 5 3 18.docx, 05.09.18 Schedule for Panelists.docx

I just spoke to Annie at the Bush Center. Originally you were part of the first panel…but they did change things and now you are on the 2nd panel. This is the final and the precall will go over this tomorrow.

Patricia L. Conrad
Special Assistant to the Director

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From: Walker, Annie (b)(6)
Sent: Thursday, May 03, 2018 4:28 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: RE: on behalf of Dr. Fauci - Please read

Here are the updated versions. I am available for the next 15 minutes.

Annie Walker
Event Assistant
George W. Bush Presidential Center
T: (b)(6) | M:
E: (b)(6)

From: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Sent: Thursday, May 03, 2018 3:18 PM
To: Walker, Annie (b)(6)
Subject: RE: on behalf of Dr. Fauci - Please read

Ok – will call you then. can you resend the final agenda that has what panel he is on?

thx

AMERICAN OVERSIGHT

Patricia L. Conrad
Special Assistant to the Director

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From: Walker, Annie (b)(6)
Sent: Thursday, May 03, 2018 4:16 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: RE: on behalf of Dr. Fauci - Please read

Hi Patty,
Yes, I am available until 3:30 p.m. my time to talk!

ANNIE WALKER
EVENT ASSISTANT

GEORGE W. BUSH PRESIDENTIAL CENTER
T: (b)(6) | E: (b)(6)

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From: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Sent: Thursday, May 03, 2018 3:13 PM

AMERICAN OVERSIGHT

To: Walker, Annie (b)(6)
Subject: on behalf of Dr. Fauci - Please read

Hi Annie –

Do you have time for a quick call? In earlier emails, Dr. Fauci was on the first panel. On the latest document you sent – it shows him on the second panel.

Can you please confirm which agenda we should use and please resend again so I make sure we have the latest and correct agenda? So sorry for any confusion – I just want to make sure we have it right.

Thank you

Patricia L. Conrad
Special Assistant to the Director

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From: Walker, Annie (b)(6)
Sent: Thursday, May 03, 2018 11:14 AM
To: (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Prince, Emma (b)(6) Sullivan, Kevin (b)(6)
Subject: RE: Event Logistics and Schedule - May 9, 2018

Mark and Dr. Fauci,
I have you both confirmed to brief with Sully tomorrow at 3:00 p.m. CST. Please review the attached document in advance of the call.

Annie Walker
Event Assistant
George W. Bush Presidential Center
T: (b)(6) | M:
E: (b)(6)

From: Walker, Annie
Sent: Wednesday, May 02, 2018 10:43 AM

AMERICAN OVERSIGHT

Cc: Prince, Emma (b)(6)
Subject: Event Logistics and Schedule - May 9, 2018

Panelists,

Thank you so much for joining us next Thursday, May 9 for our special ENGAGE Event: *The Lazarus Effect, 15 Years Later.* Attached is a speaker schedule for your reference. Could you please confirm your attendance for the dinner following the Engage program on May 9? Also, please let us know if you will have a guest, and if there are any dietary restrictions we need to be aware of for the evening. The dinner will be hosted by President and Mrs. Bush and take place in our Presidential Reception Hall immediately following the event.

Also, I have confirmed transportation to and from the hotel to the event, noted in the attached speaker schedule. If you need transportation to and from the airport, please let me know as soon as possible so I can coordinate. You are also welcome to Uber and submit your receipts to me for reimbursement.

Thank you in advance! We are looking forward to next week.

Best,
Annie Walker

ANNIE WALKER
EVENT ASSISTANT

GEORGE W. BUSH PRESIDENTIAL CENTER
T: (b)(6) E: (b)(6)

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May 3, 2018

The Lazarus Effect: 15 Years Later // Overview

May 2018 is the 15th anniversary of the President's Emergency Plan for AIDS Relief (PEPFAR), which was signed into law by President Bush. PEPFAR is credited with saving millions of lives and today supports more than 13.3 million people with lifesaving antiretroviral treatment. As President Bush wrote in his memoir, *Decision Points*, "People who were given up for dead were restored to healthy and productive lives…Africans came up with a phrase to describe the transformation. They called it the Lazarus Effect."

To celebrate the life-changing success of PEPFAR and other related programs made possible by the generosity of the American people, **Barbara Bush**, co-founder of Global Health Corps, will moderate two panels on the impact and future of global health involvement.

Panel 1: The largest international health initiative in history for a single disease/saving lives and changing the paradigm. Participants:

- Former Rwandan Minister of Health **Dr. Agnes Binagwaho**
- Key architect of PEPFAR **Ambassador Mark Dybul, M.D.**
- *Washington Post* columnist **Michael Gerson**

Key topics:

- Stories from those who were present at the birth and implementation of PEPFAR
- Themes to draw out:
  - There was bold, bipartisan leadership
  - Compassion and generosity of the American people
  - Powerful survivor stories (is there a surivivor we could have in the room?)
  - PEPFAR as the manifestation of a transformative, new paradigm - The Monterrey Consensus: moving from paternalism to partnership and service/from money spent to results achieved and good governance/from government to government to people to people, all sectors need to engage including private sector.
  - Driving entire US government approach based on data
  - First ever national scale, chronic prevention and care – opening door to all chronic disease.

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Panel 2: The state of health in Africa today…and looking forward, getting to the end of the epidemic. Participants:

- Key architect of PEPFAR **Dr. Anthony Fauci**
- Partner in Health's **Dr. Paul Farmer**
- **Favorite "Favo" Iradukunda**, former Global Health Corp Fellow from Rwanda
- **Senator Lindsey Graham** (R-South Carolina)

Key topics:

- Scientific innovation coming, we need it to get to the end
- Need to stay engaged/U.S. government leadership and funding
- Focus on adolescents and young people/girls and boys – could lose control of the epidemic that requires a new approach beyond just medical intervention
- Using the platform for person-centered care/PRRR, etc. (Note – we need to see where we are in the PRRR transition and adapt comments about PRRR)

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HHS-NIAID-20-0593-A-001046

Engage

Presented by HIGHLAND CAPITAL MANAGEMENT

The Lazarus Effect, 15 Years Later
Wednesday, May 9, 2018
George W. Bush Presidential Center

SPEAKER SCHEDULE

5:00 p.m. Premier Transportation picks up speakers at the Highland drops off at the Bush Institute
5:30 p.m. **Speaker Walk Through**
5:30 p.m. Doors open
Registration— Founding Donor Wall, Museum
Museum Tours
Cash Bar— Institute Cross Hall
Museum Pop Up Shop open in Institute Alcove
6:10 p.m. Program participants proceed to green room to receive lavalier microphone
6:25 p.m. Auditorium doors close
6:28 p.m. *Engage Video plays*

Off-stage announce of President George W. Bush

6:30 p.m. **Welcome Remarks and Introduction**
President George W. Bush

6:45 p.m. **Panel One**
Barbara P. Bush, Co-founder and Board Member, Global Health Corps
Dr. Agnes Binagwaho, Former Rwandan Minister of Health
Ambassador Mark Dybul, M.D.
Michael Gerson, Washington Post

7:25 p.m. **Panel Two**
Barbara P. Bush, Co-founder and Board Member, Global Health Corps
Dr. Paul Farmer, Partner in Health
Dr. Anthony Fauci, National Institute of Health
Senator Lindsey Graham, (R-South Carolina)
Favorite Iradukunda, Global Health Alumni

8:00 p.m. **Closing Remarks**
Ken Hersh, President and CEO, George W. Bush Presidential Center

8:05 p.m. Event Concludes

8:10 p.m. Program Participant Dinner
Presidential Reception Hall

9:30 p.m. Premier Transportation to pick up speakers at Bush Institute and drop off at Highland Hotel

Contact Information:

Annie Walker (214) 864-6246 Emma Prince (214) 675-2106 Kevin Sullivan (703) 343-5757

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Thu, 3 May 2018 15:25:55 +0000
To: Fauci, Anthony (NIH/NIAID) [E];Haskins, Melinda (NIH/NIAID) [E];Selgrade, Sara (NIH/NIAID) [E]
Subject: discuss the May 11 meeting with the Senate appropriations clerks
Attachments: RE: Pre-brief request for May 11 clerks meeting

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From: Vance, Meaghan (NIH/NIAID) [C]
Sent: Thu, 3 May 2018 12:52:06 +0000
To: Selgrade, Sara (NIH/NIAID) [E];Conrad, Patricia (NIH/NIAID) [E]
Cc: NIAID OCGR Leg
Subject: RE: Pre-brief request for May 11 clerks meeting

Can you do 3pm today?

From: Selgrade, Sara (NIH/NIAID) [E]
Sent: Wednesday, May 2, 2018 3:15 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: NIAID OCGR Leg (b)(6) Vance, Meaghan (NIH/NIAID) [C]
(b)(6)
Subject: Pre-brief request for May 11 clerks meeting

Hi Patty,

Could we have a few minutes with him this week to discuss the May 11 meeting with the Senate appropriations clerks? ASF will discuss flu and CARB with them.

Please let us know what might be convenient.

FYI - I have another pre-brief request for next week coming your way shortly…

Thanks very much for your help,
Sara

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From: Stover, Kathy (NIH/NIAID) [E]
Sent: Thu, 3 May 2018 18:03:54 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Billet, Courtney (NIH/NIAID) [E];Folkers, Greg (NIH/NIAID) [E];Conrad, Patricia (NIH/NIAID) [E]
Subject: FOR ASF REVIEW: Draft PR re: fusion peptide HIV vaccine
Attachments: Fusion peptide vax PR v6.docx, NSWB Clearance Sheet - fusion peptide vaccine.docx, Kwong manuscript.pdf

Hi Dr. Fauci,

Please find attached for your review a draft press release describing the VRC's experimental fusion peptide HIV vaccine's ability to elicit antibodies in mice, guinea pigs and monkeys that neutralized dozens of HIV strains. The findings will appear in *Nature Medicine* at some point this month.

Your quote is highlighted below:

"NIH scientists have used their detailed knowledge of the structure of HIV to find an unusual site of vulnerability on the virus and design a novel and potentially powerful vaccine," said NIAID Director Anthony S. Fauci, M.D. "This elegant study is a potentially important step forward in the ongoing quest to develop a safe and effective HIV vaccine."

Best,
Kathy

Kathy Stover
Branch Chief, News and Science Writing Branch
National Institute of Allergy and Infectious Diseases
National Institutes of Health
31 Center Drive, Room 7A17E
Bethesda, MD 20892
Direct: (b)(6)
Media Line: (b)(6)

AMERICAN OVERSIGHT

DRAFT

EMBARGOED FOR RELEASE
Day, May __, 2018
___ a.m./p.m. Eastern Time

Media Contact: Laura S. Leifman
(301) 402-1663
Email: laura.sivitz@nih.gov

HIV Vaccine Elicits Antibodies in Animals that Neutralize Dozens of HIV Strains
NIH Study Results Represent Major Advance for Structure-Based HIV Vaccine Design

An experimental vaccine regimen based on the structure of a vulnerable site on HIV elicited antibodies in mice, guinea pigs and monkeys that neutralize dozens of HIV strains from around the world. The findings were reported today in the journal *Nature Medicine* by researchers at the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health, and their colleagues.

Peter D. Kwong, Ph.D., and John R. Mascola, M.D., led the study. Dr. Kwong is chief of the Structural Biology Section at the NIAID Vaccine Research Center, and Dr. Mascola is the center director.

"NIH scientists have used their detailed knowledge of the structure of HIV to find an unusual site of vulnerability on the virus and design a novel and potentially powerful vaccine," said NIAID Director Anthony S. Fauci, M.D. "This elegant study is a potentially important step forward in the ongoing quest to develop a safe and effective HIV vaccine."

A preliminary human trial of the new vaccine regimen is anticipated to begin in the second half of 2019.

Today's report reflects one of two broad, complementary approaches NIAID is pursuing to develop an HIV vaccine. In one approach, scientists first identify powerful HIV antibodies that can neutralize many strains of the virus, and then try to elicit those antibodies with a vaccine based on the structure of the HIV surface protein where the antibodies bind. In other words, scientists start with the most promising part of the immune response and work to develop a vaccine that will induce it. This method was used to design the vaccine described today.

The other, empiric approach to HIV vaccine development begins by evaluating the most encouraging vaccine candidates for efficacy in people through clinical trials. Then scientists try

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to build on successful trial results by, for example, examining blood and other clinical specimens from study participants who received the vaccine to identify the most promising parts of the immune response. Researchers subsequently use this information to improve vaccination approaches for future trials. This method was used to develop the HIV vaccine regimen tested in the RV144 clinical trial and the HIV vaccine regimens currently under study in the HVTN 702 and Imbokodo clinical trials.

Over the past several years, HIV researchers have discovered many powerful, naturally occurring antibodies that can prevent multiple HIV strains from infecting human cells in the laboratory. About half of people living with HIV make these so-called "broadly neutralizing" antibodies, but usually only after several years of infection—long after the virus has established a foothold in the body. Scientists have identified and characterized the sites, or epitopes, on HIV where each known broadly neutralizing antibody binds. Many laboratories around the world are developing HIV vaccine candidates based on the structure of these epitopes with the goal of coaxing the immune systems of HIV-negative people to make protective antibodies after vaccination.

The experimental vaccine described in today's report is based on an epitope called the HIV fusion peptide, identified by NIAID scientists in 2016. The fusion peptide, a short string of amino acids, is part of the spike on the surface of HIV that the virus uses to enter human cells. According to the scientists, the fusion peptide epitope is particularly promising for use as a vaccine because its structure is the same across most strains of HIV, and because the immune system clearly "sees" it and makes a strong immune response to it. The fusion peptide lacks sugars that obscure the immune system's view of other HIV epitopes.

To make the vaccine, the researchers engineered many different immunogens—proteins designed to activate an immune response. These were designed using the known structure of the fusion peptide. The scientists first assessed the immunogens using a collection of antibodies that target the fusion peptide epitope, and then tested in mice which immunogens most effectively elicited HIV-neutralizing antibodies to the fusion peptide. The best immunogen consisted of eight amino acids of the fusion peptide bonded to a carrier that evoked a strong immune response. To improve their results, the scientists paired this immunogen with a replica of the HIV spike.

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The researchers then tested different combinations of injections of the protein plus HIV spike in mice and analyzed the antibodies that the vaccine regimens generated. The antibodies attached to the HIV fusion peptide and neutralized up to 31 percent of viruses from a globally representative panel of 208 HIV strains.

Based on their analyses, the scientists adjusted the vaccine regimen and tested it in guinea pigs and monkeys. These tests also yielded antibodies that neutralized a substantial fraction of HIV strains, providing initial evidence that the vaccine regimen may work in multiple species.

The scientists are now working to improve the vaccine regimen, including making it more potent and able to achieve more consistent outcomes with fewer injections. The researchers also are isolating additional broadly neutralizing antibodies generated by the vaccine in monkeys, and they will assess these antibodies for their ability to protect the animals from a monkey version of HIV. The NIAID scientists will use their findings to optimize the vaccine and then manufacture a version of it suitable for testing in human volunteers in a carefully designed and monitored clinical trial.

NIAID conducts and supports research—at NIH, throughout the United States, and worldwide—to study the causes of infectious and immune-mediated diseases, and to develop better means of preventing, diagnosing and treating these illnesses. News releases, fact sheets and other NIAID-related materials are available on the NIAID website.

About the National Institutes of Health (NIH): NIH, the nation's medical research agency, includes 27 Institutes and Centers and is a component of the U.S. Department of Health and Human Services. NIH is the primary federal agency conducting and supporting basic, clinical, and translational medical research, and is investigating the causes, treatments, and cures for both common and rare diseases. For more information about NIH and its programs, visit www.nih.gov.

###

NIH...Turning Discovery Into Health®

Reference: K Xu *et al*. Epitope-based vaccine design yields fusion peptide-directed antibodies that neutralize diverse strains of HIV-1. *Nature Medicine* DOI: _____ (2018)

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NIAID News and Science Writing Branch (NSWB)
Clearance Form

X__ News Release ___Media Availability __Statement __NIH Record
__Other (specify):

Title: HIV Vaccine Elicits Antibodies in Animals that Neutralize Dozens of HIV Strains
Audience: science media, HIV advocates, interested lay public
Distribution Date/ Embargo Date: Early to mid-May, exact date TBD
NSWB Contact/Writer: Laura Leifman
Comments:

DATE	NAME
	Scientific Reviewers:
_4/16_____	Peter Kwong
_4/17_____	John Mascola
	Division Directors:
_4/17_____	John Mascola
_4/20_____	Carl Dieffenbach
_N/A_____	Budget Officer (as needed)
_4/11____	Anne Rancourt, HIV & AIT Section Chief
_4/19_____	Kathy Stover, NSWB Branch Chief
_4/24_____	Courtney Billet, OCGR Director
_4/26_____	Bob Eisinger, Special Assistant for Scientific Projects
_4/26_____	Hillary Marston, Medical Officer/Policy Advisor
_4/26_____	Laurie Doepel, Special Assistant to the Director
_4/27_____	Carole Hudgings, Sr. Advisor to the Deputy Director
_4/26_____	Greg Folkers, NIAID Chief of Staff
_____	Hugh Auchincloss, NIAID Deputy Director
_____	Anthony S. Fauci, NIAID Director
_4/30_____	Other Reviewers: OAR

Revised: 8/14/2015

AMERICAN OVERSIGHT

Epitope-based vaccine design yields fusion peptide-directed antibodies that neutralize diverse strains of HIV-1

Kai Xu[1*], Priyamvada Acharya[1,2*], Rui Kong[1*], Cheng Cheng[1*], Gwo-Yu Chuang[1], Kevin Liu[1], Mark K. Louder[1], Sijy O'Dell[1], Reda Rawi[1], Mallika Sastry[1], Chen-Hsiang Shen[1], Baoshan Zhang[1], Tongqing Zhou[1], Mangaiarkarasi Asokan[1], Robert T. Bailer[1], Michael Chambers[1], Xuejun Chen[1], Chang W. Choi[1], Venkata P. Dandey[2], Nicole A. Doria-Rose[1], Aliaksandr Druz[1], Edward T. Eng[2], Katie Farney[1], Kathryn E. Foulds[1], Hui Geng[1], Ivelin S. Georgiev[3], Jason Gorman[1], Kurt R. Hill[1], Alexander J. Jafari[1], Young D. Kwon[1], Yen-Ting Lai[1], Thomas Lemmin[4], Krisha McKee[1], Tiffany Y. Ohr[1], Li Ou[1], Dongjun Peng[1], Ariana P. Rowshan[1], Zizhang Sheng[5,6], John-Paul Todd[1], Yaroslav Tsybovsky[7], Elise G. Viox[1], Yiran Wang[1], Hui Wei[2], Yongping Yang[1], Amy F. Zhou[1], Rui Chen[8], Lu Yang[8], Diana G. Scorpio[1], Adrian B. McDermott[1], Lawrence Shapiro[1,5,6], Bridget Carragher[2], Clinton S. Potter[2], John R. Mascola[1†] and Peter D. Kwong[1,5†]

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[4] Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, CA, 94143 USA.
[5] Department of Biochemistry and Molecular Biophysics and [6] Department of Systems Biology, Columbia University, New York, NY 10032, USA.
[7] Electron Microscopy Laboratory, Cancer Research Technology Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702, USA.
[8] GenScript USA, Piscataway, NJ 08854, USA.

[*] These authors contributed equally

[†] Correspondence to: E-mail: jmascola@nih.gov (J.R.M.) and pdkwong@nih.gov (P.D.K.)

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43

44

45 **A central goal of HIV-1-vaccine research is the elicitation of antibodies capable of**
46 **neutralizing diverse primary isolates of HIV-1. Here we show that focusing the immune**
47 **response to exposed N-terminal residues of the fusion peptide, a critical component of the**
48 **viral entry machinery and the epitope of antibodies elicited by HIV-1 infection, through**
49 **immunization with fusion peptide-coupled carriers and prefusion-stabilized envelope**
50 **trimers, induces cross-clade neutralizing responses. In mice, these immunogens elicited**
51 **monoclonal antibodies capable of neutralizing up to 31% of a cross-clade panel of 208**
52 **HIV-1 strains. Crystal and cryo-electron microscopy structures of these antibodies**
53 **revealed fusion peptide-conformational diversity as a molecular explanation for the cross-**
54 **clade neutralization. Immunization of guinea pigs and rhesus macaques induced similarly**
55 **broad fusion peptide-directed neutralizing responses suggesting translatability. The N**
56 **terminus of the HIV-1-fusion peptide is thus a promising target of vaccine efforts aimed at**
57 **eliciting broadly neutralizing antibodies.**

58
59

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HHS-NIAID-20-0593-A-001056

60 Since crossing from chimpanzees ~100 years ago[1], HIV-1 has evolved to be one of the
61 more diverse viruses to infect humans[2]. While antibodies capable of neutralizing ~50% of
62 circulating HIV-1 strains arise in half of those infected after several years[3], the vaccine
63 elicitation of antibodies capable of neutralizing divergent strains of HIV-1 remains an unsolved
64 problem: antibodies elicited by current candidate vaccines fail to neutralize more than a small
65 fraction of the diverse primary isolates that typify transmitted strains of HIV-1 (ref. [4,5]).
66 We and others have isolated broadly neutralizing antibodies from HIV-1-infected donors
67 and coupled antibody identification with structural characterization to delineate sites of
68 vulnerability to neutralizing antibodies[6,7]. These antibodies target the viral entry machinery, the
69 envelope (Env) trimer, composed of three gp120 and three gp41 subunits. Dozens of structurally
70 defined epitopes have been determined that can be categorized into a handful of Env regions.
71 The majority of identified neutralizing antibodies have characteristics that may make them
72 difficult to elict by vaccination, including those to the CD4-binding site[8,9], where extensive
73 somatic hypermutation (SHM) appears to be required[10-12], those to a quaternary site at the trimer
74 apex[13-16], where unusual recombination appears to be required[13,14,17,18], those to a glycan-V3
75 supersite[19-21], where recognition of *N*-linked glycan appears to be required[20-22], and those to the
76 membrane-proximal external region[23-26], where co-recognition of membrane[27-29] and immune
77 tolerance appear to be required[30].
78 Recently, we identified an antibody, N123-VRC34.01 (ref. [31]), named for donor (N123),
79 lineage (VRC34) and clone number (01), and hereafter referenced without donor prefix. VRC34.01
80 targets primarily the conserved N-terminal region of the HIV-1 fusion peptide (FP), a critical
81 component of viral entry machinery[32]. FP is composed of 15-20 hydrophobic amino acids at the N
82 terminus of the gp41 subunit of HIV Env and had been thought to be poorly immunogenic: hidden

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HHS-NIAID-20-0593-A-001057

83 from the immune system in the native prefusion state of Env and buried in the cell membrane after
84 Env rearranges into the postfusion state. The VRC34.01 antibody, however, revealed the N-terminal
85 half of FP to be a site of neutralization vulnerability. VRC34.01 directs the majority of its binding
86 energy to eight N-terminal residues of FP, with the rest coming from interactions with Env including
87 glycan N88 (ref. [31]). The ability to neutralize HIV-1 through recognition of a linear peptide, which is
88 both conserved in sequence and exposed in the prefusion-closed conformation of Env, suggested that
89 the site of vulnerability defined by VRC34.01 might be amenable to epitope-based approaches to
90 vaccine design. In this study we describe an antibody-to-vaccine development process. Beginning
91 with the epitope of VRC34.01, we engineered immunogens with antigenic specificity for FP-directed
92 antibodies, immunized C57BL/6 mice, and analyzed the resultant antibodies. Based on these
93 analyses, we devised 2nd-generation immunization regimens that generated improved neutralizing
94 monoclonal antibodies. We extracted insights from the murine immunizations and applied these to
95 immunize guinea pigs and rhesus macaques. Overall, these vaccine studies demonstrated the ability,
96 in multiple standard-vaccine test species, to induce serum responses capable of neutralizing a
97 substantial fraction of primary isolate strains representative of the global diversity of HIV-1.
98

99 **Fusion peptide-based immunogens**

100 The N-terminal eight residues of FP were chosen as a vaccine target, based on their
101 recognition by antibody VRC34.01 in co-crystal structures and on molecular dynamics
102 simulations, which showed these residues to be exposed and flexible in conformation[31]. To
103 obtain immunogens capable of eliciting FP-directed antibodies, we utilized structure-based
104 design to engineer FP-containing immunogens and assessed their antigenic specificity against a
105 panel of antibodies encompassing both broadly neutralizing antibodies and poorly or non-

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HHS-NIAID-20-0593-A-001058

106 neutralizing antibodies, with an emphasis on antibodies reported to engage FP as part of their
107 recognized epitope such as ACS202 (ref. [33]) and PGT151 (ref. [34]) (Fig. 1a). We used an
108 antigenicity score derived from the binding affinity of epitope-specific neutralizing and weak-
109 /non-neutralizing antibodies (see Methods) to estimate the epitope-specific suitability of each
110 immunogen (Supplementary Fig. 1a). We produced epitope scaffolds that incorporated the N-
111 terminal eight amino acids of FP, and in some cases, included added sites of *N*-linked
112 glycosylation, which we positioned analogously to FP and glycan N88 in the VRC34.01 epitope.
113 We characterized two scaffolds, which were trimeric and tetrameric from Protein Data Bank
114 (PDB) 3HSH[35], and 1SLF[36], respectively, and also assessed epitope scaffolds engineered from
115 PDB 1M6T[37] and from PDB 1Y12 (ref. [38]), which we previously described[31] (Supplementary
116 Fig. 1b-g), with the oligomeric scaffolds generally showing stronger binding to the FP-directed
117 antibodies.

118 We also created a FP-carrier protein conjugate, by coupling the eight N-terminal residues
119 of FP with an appended C-terminal cysteine to lysine residues in keyhole limpet hemocyanin
120 (KLH), a carrier protein often used to improve immunogenicity[39]. The resultant FP8-KLH was
121 robustly recognized by FP-directed antibodies and stable to extremes of temperature and
122 osmolality, and at pH 10.0, but not at pH 3.5, and negative stain-EM revealed the FP-conjugated
123 KLH to retain the barrel shape of KLH (Supplementary Fig. 1b, c, h, i). When assessed with the
124 FP-antigenicity score (Fig. 1a), FP8-KLH was superior to the FP-epitope scaffolds and similar to
125 the stabilized Env trimers, such as the SOSIP.664 (ref. [40]) or DS-SOSIP[41] trimers from the clade A
126 strain BG505.

127

128 **Immunogens with high FP-antigenic specificity induce FP-directed neutralizing responses**

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129 To assess the ability of the 1st-generation epitope-based vaccine immunogens to elicit
130 neutralizing responses, we tested immunization regimens using the two immunogens with the
131 highest FP-antigenicity scores: FP8-KLH and stabilized Env trimer. In an initial experiment, four
132 C57BL/6 mice each received 50 μg of BG505 SOSIP Env trimer and were boosted with 25 μg of
133 FP8-KLH at day 14 (Fig. 1b). After a second boost at day 28, strong FP-ELISA responses were
134 observed at day 35. After a final boost at day 49, we tested day 52 serum for neutralization of the
135 Env-pseudovirus BG505, and also of a BG505 Env variants missing glycans at positions 88 or
136 611, as these Env variants are more sensitive to FP-directed antibodies[31]. While serum
137 neutralization of wild-type BG505 generally did not pass our ID_{50} threshold for murine
138 neutralization (at least 1:40 and at least 2-fold above background), we did observe unambiguous
139 serum neutralization for the Δ88+611 glycan-deleted variant of BG505 by all four of the mice
140 (Fig. 1c, Supplementary Fig. 2a).
141 In a subsequent experiment, three mice each received 25 μg of FP-KLH, followed by
142 boosts at day 14 and day 35 (Fig. 1d). Serum ELISAs revealed Env trimer recognition in mouse
143 2586 at days 21 and 38, which also appeared in a second mouse after the third boost. We tested
144 day 38 serum for neutralization and observed neutralization for the Δ88+611 glycan-deleted
145 variant of BG505 by two of the sera (Fig. 1e, Supplementary Fig. 2a).
146

147 **First generation FP-directed antibodies neutralize up to ~10% of HIV-1 strains**

148 To provide insight into the antibodies elicited by FP-containing immunogens, we selected
149 B cell hybridomas capable of binding both BG505 SOSIP trimer and FP-1M6T scaffold, using B
150 cells from mouse 1868 (immunized with Env trimer and FP-KLH) and mouse 2586 (immunized
151 with FP-KLH only). Sequences of eight hybridomas from mouse 1868 and five hybridomas from

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152 mouse 2586 revealed seven vaccine-elicited (v) antibody lineages (vFP1-vFP7), which could be
153 segregated into three classes defined by similar B cell ontogeny and structural mode of
154 recognition[6], which we named vFP1, vFP5, and vFP6, after the first identified member of each
155 class (Supplementary Fig. 3a, b, Supplementary Tables 1-2).

156 We tested neutralization of the 12 vFP antibodies on a panel of wild-type and glycan-
157 deleted HIV-1 variants. Clear neutralization of wild-type HIV-1 strains was observed with only a
158 few of the vFP1-class antibodies (vFP1.01, vFP7.04 and vFP7.05). We assessed the three best
159 vFP1 antibodies along with antibody vFP5.01 on a well-characterized panel of 208 Env-
160 pseudoviruses[5], encompassing diverse strains of primary isolates from all of the major clades, of
161 which 154 were resistant to neutralization by CD4-induced antibodies 17b and 48d, the V3-
162 directed antibodies, 447-52D and 3074, and antibody F105, which recognized open
163 conformations of Env (Supplementary Fig. 3c, Supplementary Table 3a). Notably, vFP1.01
164 neutralized 18 strains at 50 μg/ml (8.2% breadth on 208-strain panel; 7.7% breadth on 154-
165 resistant strain panel), while vFP7.04 neutralized 20 strains at 50 μg/ml (9.6% breadth on 208-
166 strain panel; 8.3% breadth on 154-resistant strain panel). vFP5.01, by contrast, neutralized only
167 two strains, both sensitive to V3 and CD4-induced antibodies. Overall, neutralization by these 1st-
168 generation monoclonal antibodies was of limited breadth and potency, although the best vFP
169 antibodies could neutralize selected diverse strains of HIV-1.

170

171 **Disparate antibody-bound FP conformations**

172 To provide insight into the structural basis for neutralization by the 1st-generation
173 vaccine-elicited antibodies, we determined crystal structures for the antigen-binding fragment
174 (Fab) of vFP1.01 and vFP5.01 antibodies in complex with the N-terminal eight residues of FP

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175 (Ala512-Phe519) at 2.0- and 1.5-Å resolution, respectively (Supplementary Table 4a,
176 Supplementary Fig. 4, also see expanded views in Fig. 2a, b). The vFP1.01 co-crystals with FP
177 were orthorhombic with four molecules per asymmetric unit, and in all four independent copies,
178 Fab and FP assumed similar conformations, with FP adopting a curved structure, with no
179 intrachain-backbone hydrogen bonds. The N terminus of FP (Ala512) was buried between heavy
180 and light chains, with the amino terminus forming a buried salt bridge with $Glu34_{vFP1.01-LC}$,
181 which was germline encoded and shielded from solvent by a tetra-tyrosine cage, comprising
182 tyrosines at residues $27D_{vFP1.01-LC}$, $32_{vFP1.01-LC}$, $96_{vFP1.01-LC}$, and $98_{vFP1.01-HC}$ (for clarity, we
183 reference the molecule as a subscript for all molecules other than HIV Env by antibody name and
184 HC or LC for heavy or light chain, respectively). The FP-main chain paralleled the curvature of
185 the vFP1.01 CDR H3, albeit with opposite orientation, up to residue Ile515, which packed
186 against the body of the heavy chain, before extending from antibody into the main body of the
187 trimer with Gly516-Phe519 (Supplementary Fig. 4a, b).

188 The vFP5.01 co-crystals with FP were monoclinic, with one molecule per asymmetric
189 unit. vFP5.01 bound FP at the interface of heavy and light chains with the peptide adopting an
190 overall hook structure: starting with a surface-exposed Ala512, dipping into the hydrophobic
191 antibody interface with aliphatic side chains of Val513 and Ile515 anchoring the FP-N terminus,
192 before turning at Gly516, and extending from antibody towards Env (Supplementary Fig. 4c, d).

193 Comparison of antibody-bound crystal structures indicated substantial differences in FP
194 conformation (Fig. 2c, d). While antibodies VRC34.01, vFP1.01 and vFP5.01 recognized the N-
195 terminus of FP by using a similar region at the CDR H3-CDR L3 interface of each antibody, the
196 conformations of the antibody-bound FPs were substantially different: the vFP1.01-bound FP
197 formed a U-shaped structure focused at the heavy-light interface, the vFP5.01-bound FP in an

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198 extended conformation to interact with CDR H3, and the VRC34.01-bound FP in an extended
199 conformation to interact with CDR H1. To place these disparate antibody-recognized
200 conformations of FP into a more general context, we used principal component analysis to
201 cluster N-terminal FP conformations from a molecular dynamics simulation of fully glycosylated
202 HIV-1 Env. Four prevalent clusters of fusion-peptide conformations were observed
203 (Supplementary Fig. 4e-f), with FP-directed antibodies recognizing disparate but prevalent
204 conformations of FP.
205

206 **Restricted angle of approach for FP-directed neutralization**

207 To position the vFP1.01 and vFP5.01 structures with FP into the context of the HIV-1-
208 Env trimer, we collected cryo-electron microscopy (cryo-EM) data for these FP-directed
209 antibodies complexed to the BG505 SOSIP trimer. With Fab vFP1.01, approximately 14,000
210 particles yielded an 8.6-Å resolution reconstruction after three-fold averaging (Supplementary
211 Fig. 5a-e); the resulting structure (Fig. 2a) showed three Fabs laterally interacting with the Env
212 trimer. With Fab vFP5.01, several particle classes were observed yielding 14.7- and 19.6 -Å
213 resolution reconstructions (Supplementary Fig. 5f-h); these asymmetric reconstructions indicated
214 each of the vFP5.01 Fabs to approach Env differently (Fig. 2b).
215 To provide insight into recognition of the Env trimer, we analyzed the approach angle of
216 the FP-recognizing antibodies (Fig. 2e). Overall, the approach of antibodies directed primarily to
217 FP and capable of neutralizing diverse HIV-1 strains was similar, suggesting restrictions on
218 trimer approach for effective FP-directed neutralization.
219

220 **Considerations for improved immunizations**

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221 Analysis of the 1st-generation antibodies indicated effective FP-directed neutralization to
222 occur preferentially at a restricted angle of trimer approach, thereby suggesting that boosting
223 with Env trimer might elicit improved neutralization. We sought additional clues from analysis
224 of the 1st-generation vFP antibodies to improve FP immunization.

225 To provide insight into sequence requirements for neutralization, we created a panel of
226 peptides comprising Ala and Gly mutants of the N terminus of FP and screened for recognition by
227 vaccine-elicited antibodies and by VRC34.01 (Supplementary Fig. 6a, Supplementary Table 5).
228 The Ala-Gly mutants only affected vFP1.01 recognition if they occurred within the first four
229 residues of FP (512-515). For vFP5.01, a more extensive range was observed, with alterations to
230 Ala-Gly at residues 513, 514, 515, 516, and 519 affecting recognition. VRC34.01 recognition by
231 comparison was intermediate between vFP1.01 and vFP5.01, being sensitive to changes at 513,
232 515, and 516, and partially sensitive to changes at 518 and 519. These results indicated a
233 preference for N-terminal residues for effective neutralization, thereby suggesting that N-terminal
234 focusing might improve neutralization of the vaccine-elicited antibodies.

235 Although we did not observe a significant improvement in neutralization titers upon Env
236 priming (Fig. 1), we nevertheless analyzed the degree of affinity maturation for vFP1-class
237 antibodies as this would lend insight into the induction of these antibodies. We identified vFP1-
238 class antibodies from three additional mice, two of them (1882 and 1883), primed with Env trimer,
239 and one (2602), immunized with only FP-KLH (Supplementary Fig. 7a). Notably, vFP1-class
240 antibodies primed with Env trimer showed higher somatic hypermutation (SHM) with statistically
241 significance (Supplementary Fig. 7b). Thus, while Env-trimer priming did not improve
242 neutralization, it did appear to prime vFP1-class antibodies.
243

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244 **Second generation FP-directed antibodies neutralize up to 31% of HIV-1 strains**

245 To elicit improved FP-directed antibodies, we tested 11 immunization regimens on 16
246 C57BL/6 mice (Supplementary Fig. 8a). The vaccine regimens included a BG505 SOSIP-trimer
247 prime and various FP-KLH boosts, along with a BG505 DS-SOSIP boost on a subset of five of the
248 mice. The FP-KLH boosts utilized different lengths of FP, ranging from FP6 through FP10, which
249 incorporated 6 through 10 residues from the N-terminal FP sequence of strain BG505. Serum
250 neutralization titers for the five trimer-boosted animals were especially improved, reaching an ID_{50}
251 as high as 77,379 (mouse 2716) for the glycan deleted (Δ88+611) BG505, (Supplementary Fig.
252 8a,b). We tested the serum neutralization for the 5 trimer-boosted mice on a panel of 10 selected
253 VRC34-sensitive wild-type strains, which encompassed divergent HIV-1 clades (Supplementary
254 Figs. 2b and 8c). While modest neutralization from these five sera was observed against many of
255 the selected strains, serum from mouse 2716 achieved ID_{80} neutralization against most of these
256 viruses. Analysis by peptide competition indicated neutralization by the mouse 2716 serum to be
257 targeted primarily to FP (Supplementary Fig. 2c).

258 For each of the 16 immunized C57BL/6 mice, we isolated and characterized monoclonal
259 antibodies. These could be parsed into 21 lineages, vFP12-vFP32 (Supplementary Table 1a-c), and
260 we screened each of the vFP antibodies against two wild-type viruses, the clade A BG505 and the
261 clade B 3988.25; 24 antibodies showed wildtype neutralization, with most neutralizing 70% or
262 more of the 10-strain panel (Supplementary Table 3b).

263 We chose two antibodies, 2712-vFP16.02 and 2716-vFP20.01, for in depth assessment
264 (vaccine-elicited FP antibodies were named for mouse ID-lineage.clone, with antibody 2716-
265 vFP20.01 being clone 01 from lineage vFP20 isolated from mouse ID 2716). Notably, on the 208-
266 strain panel, these two antibodies achieved 31% and 27% neutralization breadth, respectively,

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267 when assessed at a maximum IC_{50} level of 50 μg/ml (Fig. 3, Supplementary Table 3a). The breadth
268 and potency of these two vFP antibodies were similar to that of antibody 2G12 (Supplementary
269 Fig. 11b), which has been shown to delay rebound and to induce selection pressure on the viral
270 quasispecies when passively infused[42-44]. Neutralization extended to multiple clades, including
271 clades A, B, and C, but for several clades, including clades AC, AG, D, CD, and G, no
272 neutralization was observed. With the neutralized clades, the immunized FP8 sequence was
273 generally present, whereas with the non-neutralized clades, this FP8 sequence was not present
274 (with clade AE, the prevalent FP8 sequences included changes in the C-terminal region of FP8
275 (Supplementary Table 6), which were apparently tolerated by both vFP16.02 and vFP20.01
276 antibodies). We observed strong correlation between FP-directed antibody breadth on the 208-
277 strain panel and the 58-strain panel with identical FP8 sequences, indicating overall breadth of the
278 vFP antibodies to correlate with the ability to neutralize strains with the most prevalent FP8
279 sequence (Fig. 3, lower panels). These results provide proof-of-principle for the ability of FP-
280 epitope focusing to induce FP-directed antibodies with promising neutralization breadth.
281

282 **Structures and Env interactions of 2nd-generation antibodies, vFP16.02 and vFP20.01**

283 To gain insight into the cross-clade breadth observed with vFP16.02 and vFP20.01
284 antibodies, we determined their crystal structures in complex with FP (Supplementary Fig. 4g, h)
285 and their cryo-EM structures in complex with HIV-1 Env trimer (Fig. 4). The crystal structures
286 with FP revealed highly similar recognition of residues 512-517, with the vFP1-class antibodies
287 constraining little of the FP conformation beyond residue 517 (Supplementary Fig. 4g, h,
288 Supplementary Table 4a). The cryo-EM data were collected on a quaternary complex with BG505
289 DS-SOSIP trimer bound by antibodies PGT122 and VRC03, in addition to vFP16.02 or vFP20.01;

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290 the added antibodies increased the size of the complex and provided fiducial markers allowing
291 better particle visualization and alignment. The resultant reconstructions displayed resolutions of
292 3.6- and 3.8-Å, respectively, when calculated according to the FSC 0.143 gold-standard criterion
293 with soft-edged masks from which flexible constant regions had been removed (Fig. 4,
294 Supplementary Fig. 9a-f, Supplementary Table 4b). In the reconstructions, we observed electron
295 density for the vFP16.02 and vFP20.01 antibodies focused around the fusion peptide, with density
296 becoming weaker, farther from their fusion peptide site of recognition (Supplementary Fig. 10a-b).
297 Glycan contacts between vFP antibodies and Env trimer were observed (Fig. 4c, d), and
298 SHM was observed to occur preferentially at the interface with Env, especially involving
299 interactions with FP and with *N*-linked glycan (Fig. 4e). In both antibody-Env complexes, glycans
300 N448 and N611 displayed similar orientations, with glycan N448 buttressed by the light chain on
301 one side and by glycan N295 on the other and with glycan N611 projecting from a neighboring
302 Env protomer directly toward the antibody heavy chain. Glycan N88 also displayed ordered
303 density for its protein-proximal sugars, though this differed in the two antibody complexes: in
304 vFP16.02, substantial ordering was observed, with the glycan lodged between gp41 and the heavy
305 chain (Fig. 4c); in vFP20.01, glycan N88 was less ordered and assumed a substantially different
306 conformation to accommodate the SHM-altered $Gly56Tyr_{vFP20.01\text{-}HC}$ side chain (Fig. 4d). We note
307 that even though removal of glycans neighboring FP (for example, glycan 611) improved
308 neutralization by these antibodies, resistance analysis (Supplementary Table 7) indicated
309 neutralization in the 208-strain panel to not be dependent on the absence of FP-neighboring
310 glycans. Overall, the structures indicated murine vFP antibodies with promising breadth to
311 substantially accommodate, if not partially recognize, FP-proximal *N*-linked glycan.
312

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313 **Translation of murine vaccine regimens into guinea pigs and NHPs**

314 We extracted insights from the murine immunization regimens, such as trimer boost and FP
315 N-terminal focusing, and applied these to guinea pigs and rhesus macaques (non-human primates;
316 NHPs). We immunized five guinea pigs using a scheme modeled on the mouse 2716 immunization
317 regimen (Fig. 5a). At week 28, we observed 4 of 5 guinea pigs to show some heterologous virus
318 neutralization on the 10-strain panel. When compared to DS-SOSIP alone immunized guinea pigs
319 (Supplementary Fig. 7e), the 10-strain breadth induced by FP-KLH prime DS-SOSIP boost was
320 significantly higher than induced by DS-SOSIP alone (Fig. 5a, b).

321 For NHP, we also used a regimen similar to that used for mouse 2716, but omitting the
322 initial trimer prime and using three trimer boosts, so that we could compare to NHPs immunized
323 with trimer only. We used a total of 5 FP-KLH primes with FP peptides of lengths 8-8-8-7-6. At
324 week 46, we observed 4 of 5 NHP plasma to show heterologus virus neutralization on the 10-strain
325 panel (Fig. 5c, d). As seen in the GP immunization study, this breadth of neutralization by NHP
326 plasma was higher than that induced by immunization with DS-SOSIP alone (Supplementary Fig.
327 7f)[45].

328 We further assessed all 5 NHP week 46 plasma on a 58-strain subset of the 208-strain
329 panel, restricted to strains for which the sequence of FP was AVGIGAVF, the most prevalent FP
330 sequence and the sequence in the FP8-KLH and BG505 DS-SOSIP immunogens. At a 1:20
331 dilution, 3 of 5 NHP plasma showed substantial neutralization breadth, with the broadest, NHP
332 DF1W, achieving ID_{50} neutralization on 41 of 58 strains (70%) and ID_{80} neutralization of 13 of 58
333 strains (22%) (Fig. 6a, Supplementary Table 3c). Neutralization competition of the NHP DF1W
334 plasma tested against neutralization-resistant HIV-1 strains with complete glycan around FP from
335 four different clades indicated that a linear FP, matching the immunogen sequence, could adsorb

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336 virtually all of the neutralizing activity (Fig. 6b, Supplementary Fig. 2d). A dendrogram of these
337 58-Env strains (Fig. 6c) indicated neutralization to be distributed over several clades, consistent
338 with the distribution of the immunized FP8 sequence in HIV-1. Additionally, the neutralization
339 fingerprints of the three NHP plasma with substantial breadth clustered next to the neutralization
340 fingerprints of the murine vFP1-class antibodies (Fig. 6d), providing a strong indication for the
341 similarity of induced murine and NHP immune responses.
342

343 **Discussion**

344 The vaccine elicitation of antibodies capable of neutralizing diverse strains of HIV-1 has
345 been a goal of HIV-1 research for over 30 years. While substantial strides have been made in the
346 creation of prefusion-stabilized Env trimers[40,41,46-49], responses elicited by these trimers have been
347 primarily strain-specific. Here, we show that focusing the immune response to the exposed N-
348 terminal residues of FP elicits antibodies of promising neutralization breadth in multiple vaccine-
349 test species. Several factors contributed to the successful elicitation of neutralizing antibodies.
350 First, antibody VRC34.01 (ref. [31]) defined a precise target – not the entire FP, but only exposed N-
351 terminal residues – upon which to focus. Second, glycan-deleted viruses[50] provided a readout
352 sensitive enough to detect initial weak serum responses. Third, we found prefusion-closed Env
353 trimers[40] to be necessary for boosting serum responses. Most important, however, may be the
354 characteristics of the target site – the FP N-terminus – a conserved and exposed site of
355 vulnerability, for which high conformational variability was compatible with broad neutralization
356 (Supplementary Figs. 4e and 11c-g).

357 FP-directed antibodies of substantial breadth have been reported for other viral pathogens,
358 including influenza A virus[5152], Ebola virus[53-55] and Lassa virus[56]. Although the fusion peptides of

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359 these pathogens are substantially different from that of HIV-1, the generality of FP-targeted
360 neutralizing antibodies suggests that characteristics of FP may be especially suited to vaccine
361 targeting. Such characteristics include its high conservation (likely related to function), its
362 chemistry (hydrophobic residues afford high binding energy), and its exposure (FP is often
363 proximal to a site of proteolytic activation).

364 Is there a neutralization limit or constraint for vFP antibodies based on viral properties (e.g.
365 related to strain-specific conformation or accessibility) other than the diversity of FP itself? Strong
366 correlation was observed between Env trimer affinity and neutralization breadth (Supplementary
367 Figs. 6b, c, 11d), suggesting that enhanced Env-trimer affinity would lead to increased breadth.
368 Indeed, at 10-fold higher concentration (500 μg/ml), vFP16.02 neutralized 97% (56 of 58 strains)
369 with the immunized FP8 sequence, and 47% of the 208-strain panel (97 of 208 strains)
370 (Supplementary Table 3a). Thus, for strains with the same sequence as used in the FP
371 immunizations, there did not appear to be an intrinsic limit to elicited FP-directed breadth.

372 Finally, is the observed elicitation of vFP-neutralizing antibodies in animal models
373 translatable to humans? While broadly neutralizing antibodies against HIV-1 have been induced in
374 animals such as cows[57] and llamas[58], these antibodies rely on species-specific characteristics[59,60]
375 (knob domains for cow and unpaired heavy chains for llama), which are not present in human
376 antibodies. By contrast, there does not appear to be an intrinsic barrier to eliciting vFP antibodies
377 in humans: genetic analysis indicates humans to have V-genes with some similarity to the germline
378 genes of the murine vFP1-antibody class (Supplementary Fig. 12a, b); FP-directed antibodies can
379 often be detected in HIV-1-infected donors by ELISA[31]; and FP-directed antibodies showed almost
380 no indication of polyreactivity (Supplementary Fig. 12c, d). Overall, our results provide proof-of-
381 principle for the ability of epitope-based vaccine design to induce FP-directed antibodies with

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382 neutralization breadth and indicate the exposed N terminus of FP to be a site of exceptional HIV-1
383 vaccine promise.
384
385
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406

407 AUTHOR CONTRIBUTIONS

408 K.X. conceived and led the project, as well as determined all crystal structures; P.A. determined
409 cryo-EM structures; R.K. and N.A.D.-R. coordinated neutralization assessments; C.C.
410 coordinated guinea pig and NHP immunization; G.-Y.C. coordinated statistical and
411 bioinformatical analysis; K.L. prepared proteins and co-determined crystal structures; M.K.L.
412 and R.T.B. assessed antibody neutralization in the large panel; C.-H.S. performed antibody
413 sequence analysis; M.S. performed all Alanine/Glycine scans; B.Z. prepared antibodies for large
414 panel, and performed various binding analysis; T.Z. performed SPR analysis, calculated antibody
415 approaching angel to trimer and made antibody neutralization dendrograms; M.A. performed
416 antibody autoreactivity test; I.S.G. performed antibody neutralization finger-print analysis; T.L.
417 performed molecular dynamics analysis; S.D., K.M., C.W.C., E.G.V. and A.P.R. co-performed
418 neutralization assay; A.D., D.P., B.Z. and Y.Y. helped with protein expression; E.T.E., V.P.D.
419 and H.W. helped with cryo-EM structures; X.C., H.G., J.G., M.S., and Y.D.K. performed protein
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421 assisted with X-ray crystal datasets processing; B.Z., L.O. and M.C. helped with immunogen
422 preparation and characterization; R.R. and K.F. performed statistical and bioinformatical
423 analysis; Z.S. performed antibody gene comparison; Y.T. performed negative-stain EM; T.Y.O
424 participated in immunogen binding test; A.F.Z. helped with SPR assay; R.C. and L.Y. supervised
425 the research team in GenScript; A.B.M supervised MSD-based immunogen antigenicity
426 characterization; L.S. supervised antibody gene analysis and comparison; B.C. and C.S.P.
427 supervised cryo-EM studies; K.X., L.S., J.R.M. and P.D.K. wrote the manuscript, and all authors
428 read, edited and approved the manuscript. J.R.M. and P.D.K. supervised the study.

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566
567 **METHODS**

568 **Peptide synthesis and peptide-carrier protein conjugate preparation.** HIV-1 fusion peptides (FPs) were
569 synthesized (GenScript) with a free amine group on the N terminus. FP His-tagged peptides were synthesized
570 (GenScript) with a six histidine residues tag at the C terminus of FP. To prepare peptide-carrier protein conjugates,
571 each peptide with a cysteine residue added to the C terminus was conjugated to the carrier protein keyhole limpet
572 hemocyanin (KLH) (Thermo-Scientific) using m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) following
573 the manufacturer's protocol.

574 **Protein expression and purification.** BG505 SOSIP, BG505 DS-SOSIP and their glycan-deficient variants were
575 expressed and purified as described previously[41]. FP-epitope scaffold proteins, including FP-1M6T, FP-1Y12, FP-
576 3HSH and FP-1SLF were expressed and purified as described previously[31]. vFP1.01, vFP7.04, vFP16.02 and
577 vFP20.01 antibodies used for structure determination were prepared as below. Heavy chain plasmids, encoding the
578 chimera of mouse variable domain and human constant domain, with HRV3C cleavage site in the hinge region; and
579 light chain plasmids, encoding the chimera of mouse variable domain and human constant domain were co-
580 transfected in Expi293F cells (Thermo Fisher) using Turbo293 transfection reagent (SPEED BioSystem) according
581 to the manufacturer's protocol. Transfected cells were incubated in shaker incubators at 120 rpm, 37°C, 9% CO_2
582 overnight. On the second day, one tenth culture volume of AbBooster medium (ABI scientific) was added to each
583 flask of transfected cells and cell cultures were incubated at 120 rpm, 33°C, 9% CO_2 for an additional 5 days. 6 days
584 post-transfection, cell culture supernatants were harvested. IgGs were purified from the supernatants using protein A
585 chromatography: after PBS wash and low pH glycine buffer elution, the eluate was immediately neutralized using
586 10% volume of 1M Tris buffer pH 8.0. Fabs were obtained either by HRV3C cleavage or papain digestion. The
587 fragmented Fabs were further purified by size exclusion chromatograph (SEC) in a Superdex 200 column (GE) with
588 a buffer containing 5mM HEPES, pH 7.5, 150mM NaCl.

589 **Negative-stain electron microscopy.** Samples were diluted with a buffer containing 20 mM HEPES, pH 7.0, 150
590 mM NaCl, adsorbed to a freshly glow-discharged carbon-film grid, washed with the above buffer, and stained with
591 0.7% uranyl formate. Images were collected semi-automatically at a magnification of 100,000 using SerialEM[61] on a
592 FEI Tecnai T20 microscope equipped with a 2k x 2k Eagle CCD camera and operated at 200 kV. The pixel size was
593 0.22 nm/px. Particles were picked manually using the swarm mode in e2boxer from the EMAN2 software
594 package[62]. Reference-free 2D classification was performed using EMAN2 and SPIDER[63].

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595 **Antigenic characteristics of fusion peptide immunogens with Biolayer Interferometry and MSD-ECLIA.**
596 Antigenic characteristics of KLH-coupled fusion peptide, FP scaffolds and BG505 Env trimers to various FP-
597 targeting and Non-FP-targeting HIV antibodies were assessed with Biolayer Interferometry on an Octet RED384
598 (ForteBio) instrument and with MSD-ECLIA as previously described[41].
599 **Antigenicity score.** Antigenicity scores for the FP immunogens were calculated according to the metric defined in
600 Supplementary Fig. 1a. This metric sums the average binding of neutralizing antibodies versus poorly/non-
601 neutralizing antibodies, with binding defined as a function of the logarithm of antigen-binding affinity relative to
602 upper and lower binding limits. This summation is weighted by the site targeted. An antigenicity score of 1for an FP
603 immunogen therefore indicates both high specificity and tight affinity for FP-directed neutralizing antibodies; An
604 antigenicity score of 0 for an FP immunogen would indicate either low specificity or weak affinity for FP-directed
605 neutralizing antibodies. In Fig. 1a, the antigenicity score was calculated with only FP-directed antibodies; we would
606 anticipate the FP-antigenicity score for trimer immunogens to decease relative to FP-specific immunogens such as
607 FP8-KLH if non-FP-directed antibodies were considered.
608 **Mouse immunization (GenScript).** Female mice (C57BL/6) around 8 weeks old were immunized in two-week
609 intervals with either HIV-1 Env trimer or FP-KLH, using either Adjuplex as adjuvant (Sigma-Aldrich Inc, MO) for
610 trimer or GS-adjuvant (GenScript) for FP-KLH. 50 μg of immunogens were used for prime immunization and 25 μg
611 immunogens were used in boost immunization. Intraperitoneal (IP) route was used for all mice immunizations. Sera
612 samples were collected either 7 days or 14 days after each immunization for ELISA and other analyses.
613 All experiments were performed in accordance with protocols reviewed and approved by the Genscript's
614 Institutional Animal Care and Use Committee (IACUC, #ANT14-003 and #ANT17-003). All mice were housed and
615 cared for in a facility in GenScript accredited by Association for Assessment and Accreditation of Laboratory
616 Animal Care International (AAALAC International).
617 **Hybridoma creation and monoclonal antibody production.** Terminal boosts were performed on the top
618 responders from each immunization scheme as assessed with ELISA against the immunogens, three weeks after the
619 last immunization. Mice spleens were harvested three days post terminal boost, and hybridomas were generated for
620 monoclonal antibody selection following the standard procedure at GenScript. Monoclonal antibody selection was
621 based on affinity to FP-scaffold (FP-1M6T) and BG505 Env trimer as measured by ELISA.

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622 **Guinea pig and NHP protocols and immunizations.** For immunization studies, all animals were housed and cared
623 for in accordance with local, state, federal, and institute policies in an American Association for Accreditation of
624 Laboratory Animal Care-accredited facility at the Vaccine Research Center, NIAID, NIH or at a contract facility
625 (Bioqual Inc, MD). All animal experiments were reviewed and approved by the Animal Care and Use Committee of
626 the Vaccine Research Center, NIAID, NIH, and covered under protocol VRC-13-431, VRC-16-667.

627 Female Hartley guinea pigs with body weights of 300 grams were purchased from Charles River Laboratories,
628 MA. For each immunization, 400 µl of immunogen mix, containing 25 µg of specified, filter-sterilized protein
629 immunogen and 80 µl of Adjuplex (Sigma-Aldrich Inc, MO or Adjuplex equvalent formulated at VRC) in PBS, was
630 injected into the muscle of the two hind legs. While animals were under anesthesia, blood was collected through
631 retro-orbital bleeding for serological analyses.

632 Female and male Indian origin rhesus macaques with body weights of 2-9 kg were used for immunization
633 studies. For each immunization, 1 ml of immunogen mix, containing 100 µg of specified, filter-sterilized protein
634 immunogen and 200 µl of Adjuplex (Sigma-Aldrich Inc, MO or Adjuplex equvalent formulated at VRC) in PBS,
635 was injected via a needle syringe into the caudal thighs of the two hind legs. Blood was collected two weeks post
636 immunization for serological analyses.

637 **ELISA.** Fusion peptide ELISAs: Costar® High Binding Half-Area 96-well plates (Corning, Kennebunk, ME) were
638 coated with 50 µl/well of 2 µg/ml scaffold proteins in PBS overnight at 4°C. Between steps, plates were washed 5
639 times with PBS-T (PBS + 0.05% Tween) and incubated at 37°C for 1 hour. After coating, plates were blocked with
640 100 µl/well of blocking buffer (B3T: 150 mM NaCl, 50 mM Tris-HCl, 1 mM EDTA, 3.3% fetal bovine serum, 2%
641 bovine albumin, 0.07% Tween 20, 0.02% Thimerosal). 2-fold serial dilution of 1:100 pre-immunization and 1:1000
642 post immunization sera were used in the ELISA. Goat anti-mouse IgG (HRP-conjugated, GenScript) at 1:5000
643 dilution was used for detection. Plates were developed with tetramethylbenzidine (TMB) substrate (SureBlueTM,
644 KPL, Gaithersburg, MD) for 10 minutes before adding 1 N sulfuric acid (Fisher Chemical) to stop the reaction.
645 Plates were read at 450 nm (Molecular Devices, SpectraMax using SoftMax Pro 5 software) and the optical densities
646 (OD) were recorded.

647 BG505 SOSIP D7324 Capture ELISAs: Costar® High Binding Half-Area, 96-well plates (Corning,
648 Kennebunk, ME) were coated with 50 µl/well of 2 µg/ml of sheep D7324 antibody (AALTO Bio Reagents) in PBS
649 overnight at 4°C. Between steps, except for addition of trimer, plates were washed 5 times with PBS-T (PBS +

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650 0.05% Tween) and incubated at room temperature (RT) for 1 hr. After coating, plates were blocked with 100 µl/well
651 of blocking buffer (5% Skim Milk, 2% bovine albumin, 0.1% Tween 20 in TBS). Next, 50 µl/well of 0.5 µg/ml
652 D7324-tagged BG505.SOSIP trimer diluted in 10% fetal bovine serum in PBS was added and incubated at RT for 2
653 hours2-fold serial dilution of 1:100 pre-immunization and 1:1000 post immunization sera were used in the ELISA.
654 Goat anti-mouse IgG (HRP-conjugated, GenScript) at 1:5000 dilution was used for detection. Plates were developed
655 with tetramethylbenzidine (TMB) substrate (SureBlueTM, KPL, Gaithersburg, MD) for 10 minutes before adding 1
656 N sulfuric acid (Fisher Chemical) to stop the reaction. Plates were read at 450 nm (Molecular Devices, SpectraMax
657 using SoftMax Pro 5 software) and the optical densities (OD) were recorded.
658 ELISA responses were plotted using PRISM (PRISM 7 GraphPad Software for Mac OS X).
659 **Genetic assignment of antibodies.** Antibody sequences were submitted to the ImMunoGeneTics information
660 system® (IMGT, http://www.imgt.org) and subjected to variable(V), diverse(D) and joining(J) genes identification
661 by alignment with the mouse germline sequences of the IMGT reference directory, and IMGT/JunctionAnalysis for
662 a detailed analysis of the V-J and V-D-J junctions. We only considered confirmed functional germline genes for the
663 assigned germline. Clustal Omega software was used to prepare multiple sequence alignment of antibody sequences
664 for maximum likelihood phylogenetic tree construction using DNAML program in the PHYLIP package version
665 3.69 (http:// evolution.genetics.washington.edu/phylip.html). Calculations were performed based on empirical base
666 frequencies with transition/transversion (Ti/Tv) ratio of 2.0. Dendroscope 3 (dendroscope.org) was used to visualize
667 phylogenetic trees[64]. The amino acid sequence alignments were visualized using BioEdit v7.2.5 editing software[65].
668 To calculate the minimal mutations required to switch between two different unmutated common ancestors, the
669 unmutated common ancestor sequence was prepared by reverting the assigned V(D)J gene sequences into their
670 corresponding germline sequences. Differences between unmutated common ancestor sequences were counted as
671 the minimal mutations required to switch from one unmutated common ancestor to another.
672 **Antibody alanine/glycine scan.** Binding of the vaccine elicited mouse vFP antibodies to sixteen His-tagged fusion
673 peptide (residue 512-521), including wildtype and alanine/glycine mutants, was assessed using a fortéBio Octet
674 Red384 instrument. Briefly, the sixteen peptides at 50 µg/ml in PBS were loaded onto Ni-NTA biosensors using
675 their C-terminal histidine tags for 60 s. Typical capture levels were between 1.1 and 1.3 nm and variability within a
676 row of eight tips did not exceed 0.1 nm. These peptide-bound biosensors were equilibrated in PBS for 60 s followed

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677 by capture of the antigen binding fragments (Fabs, 250 nM) of the vaccine elicited vFP antibodies, VRC34.01 and
678 an RSV F antibody Motavizumab for 120 s and a subsequent dissociation step in PBS.
679 In all Octet measurements, parallel correction to subtract systematic baseline drift was carried out by
680 subtracting the measurements recorded for a loaded sensor incubated in PBS. Data analysis was carried out using
681 Octet software, version 9.0. The normalized responses obtained from one or triplicate dataset were plotted using
682 PRISM (PRISM 7 GraphPad Software for Mac OS X).
683 **Surface plasmon resonance assay.** Binding affinities and kinetics of antibodies to HIV-1 DS-SOSIP trimers and
684 His-tagged fusion peptide were assessed by surface plasmon resonance on a Biacore T-200 (GE Healthcare) at 25
685 °C. To test antibody binding with HIV-1 DS-SOSIP trimers, 2G12 IgG was first immobilized on flow cells of a
686 CM5 chip at ~3000-8000 response unit. BG505 DS-SOSIP trimer and its glycan-deleted mutants, BG505 DS-
687 SOSIP.Δ88 and BG505 DS-SOSIP.Δ611, at 500 nM in HBS-EP+ buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 3
688 mM EDTA and 0.05% surfactant P-20) were then captured onto 2G12 of one flow cell by flowing the protein
689 solution for 60 s at a flow rate of 6 µl/min. Binding affinities for the 2G12-captured trimer were determined by using
690 a serial dilution of antibody Fab solutions starting at 200nM during association phase. A dissociation phase at 30
691 µl/min for 300 s was used to determine binding kinetics. The surface was regenerated by flowing 3M $MgCl_2$
692 solution for 30 s at a flow rate of 50 µl/min. Blank sensorgrams were obtained by injection of the same volume of
693 HBS-EP+ buffer in place of antibody Fab solution. Sensorgrams of the concentration series were corrected with
694 corresponding blank curves and fitted globally with Biacore T200 evaluation software using a 1:1 Langmuir model
695 of binding.
696 Affinity of antibody Fab to the His-tagged fusion peptide was measured on a Ni-NTA sensor chip (GE
697 Healthcare). The Ni-NTA surface was activated by injection of 5 mM of Ni_2SO_4 in HBS-P+ buffer (10 mM HEPES,
698 pH 7.4, 150 mM NaCl and 0.05% surfactant P-20) for 60 s at 6 µl/min and then stabilized by washing with HBS-
699 EP+ buffer containing 3 mM EDTA for 60 s at 30 µl/min. Fusion peptide with His-tag at 20 ng/ml was captured at 6
700 µl/min flow rate for 60 s over the nickel activated sensor surface. Binding affinities for the 2G12-captured trimer
701 were determined by using a serial dilution of antibody Fab solutions starting at 200nM during association phase. A
702 dissociation phase at 30 µl/min for 300 s was used to determine binding kinetics. The surface was regenerated by
703 flowing 300 mM imidazole to both channels at 6 µl/min for 60 s. Sensorgrams of the concentration series were

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704 corrected with corresponding blank curves and fitted globally with Biacore T200 evaluation software using a 1:1
705 Langmuir model of binding.
706 **HIV-1 Env mutagenesis.** Site-directed mutagenesis on HIV-1 Env plasmids was performed through GeneImmune
707 Biotechnology LLC, NY. T90A and S613A mutations were created to remove glycan 88 and 611, respectively.
708 **HIV-1 Env-pseudotyped virus.** 293T-grown HIV-1 Env-pseudotyped virus stocks were generated by
709 cotransfection of the wildtype or mutant Env expression plasmids with a pSG3ΔEnv backbone[11].
710 **Neutralization assays.** A single round of entry neutralization assays using TZM-bl target cells were performed to
711 assess monoclonal antibody neutralization as described[11]. Briefly, the monoclonal antibodies were tested via 5-fold
712 serial dilutions starting at up to 500 μg/ml. Monoclonal antibodies were mixed with the virus stocks in a total
713 volume of 50 μl and incubated at 37 °C for 1 hr. 20 μl of TZM-bl cells (0.5 million/ml) were then added to the
714 mixture and incubated at 37 °C overnight. 130 μl cDMEM was added on day 2, and cells were lysed on day 3 and
715 assessed for luciferase activity (RLU). The 50% and 80% inhibitory concentrations (IC_{50} and IC_{80}) were determined
716 using a hill slope regression analysis as described[11].
717 To assess monoclonal antibody neutralization on a panel of 208 HIV-1 Env-pseudotyped viruses an automated
718 384-well microneutralization assay was performed as described previously[66].
719 Serum neutralization was also assessed in the single round of entry neutralization assays using TZM-bl target
720 cells, as described above. Before evaluation, all sera from immunized and control animal were heat-inactivated at 56
721 °C for 1 hr. All sera were tested via 4-fold serial dilutions starting at 1:20 dilution.
722 Serum neutralization with FP competition was performed with serum in presence of either PEGylated FP9
723 (AVGIGAVFL) or PEGylated non-cognate FLAG peptide. Mean and standard deviation (SD) of results from
724 triplicated experiments were determined.
725 **Protein complex preparation.** Antibody Fab and fusion peptide (residue 512-518) complexes were prepared by
726 first dissolving fusion peptide in 100% DMSO at 50 mg/ml concentration, then mixing with Fab solution in 10:1
727 molar ratio to reach final protein complex centration of 15 mg/ml.
728 **Protein Crystal screening.** Antibody Fab and fusion peptide (residue 512-518) complexes were screened for
729 crystallization from JCSG1-4 protein crystal screening kits using a Cartesian Honeybee crystallization robot as
730 described previously[15] and a mosquito robot. Crystals initially observed from the wells were manually reproduced.
731 vFP1.01/FP complex crystal grew in 0.2 M $AmSO_4$, 0.1 M NaOAc pH 4.6; vFP7.04/FP complex crystal grew in 0.1

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732 M MES pH 6.0, 30% PEG 6000; vFP16.02/FP complex crystal grew in 0.1 M NaOAc pH 4.5, 2 M $AmSO_4$;
733 vFP20.01/FP complex crystal grew in 0.1 M Citric acid pH 3.5, 2 M $AmSO_4$; vFP5.01/FP complex crystals grew in
734 0.2 M $MgCl_2$, 0.1 M Tris-HCl pH 8.5, 20% PEG 8000.

735 **X-ray data collection, structure solution, model building and refinement.** Crystals were cryoprotected in 20%
736 glycerol and flash-frozen in liquid nitrogen. Data were collected at a temperature of 100K and a wavelength of 1.00
737 Å at the SER-CAT beamline ID-22 (Advanced Photon Source, Argonne National Laboratory). Diffraction data were
738 processed with the HKL2000 suite[67]. Structure solution was obtained by molecular replacement with Phaser using
739 homologous Fab structures (PDB ID: 3BKY for vFP1-class antibody complex and 3LEY for vFP5.01 antibody
740 complex) as search models. Model building was carried out with Coot[68]. Refinement was carried out with Phenix[69].
741 Ramachandran statistical analysis indicated that the final structures contained no disallowed or no more than 0.23%
742 disallowed residues. Data collection and refinement statistics are shown in Supplementary Table 4.

743 **Cryo-EM data collection and processing.** Env trimer used in cryo-EM was generated in GNTI- cell line as
744 described previously[41]. To prepare Env complexes, BG505 DS-SOSIP at a final concentration of 0.3-0.5 mg/ml was
745 incubated with 4-5–fold molar excess of the antibody Fab fragments for 30-60 minutes. To prevent aggregation
746 during vitrification, the sample was incubated in 0.085 mM dodecyl-maltoside (DDM). The vFP1.01 and vFP5.01
747 bound complexes were vitrified by applying 3 µl of sample to freshly plasma-cleaned C-flat holey carbon grids (CF-
748 1.2/1.3-4C) (EMS, Hatfield, PA) for vFP1.01 and gold grids for vFP5.01, allowing the sample to adsorb to the grid
749 for 60 s, followed by blotting with filter paper and plunge-freezing into liquid ethane using the CP3 cryo-plunger
750 (Gatan, Inc.) (20°C, 85-90% relative humidity).

751 The vFP16.02 and vFP20.01 bound complexes were vitrified using a semi-automated Spotiton V1.0 robot[70]
752 The grids used were specially designed Nanowire self-blotting grids with a Carbon Lacey supporting substrate.
753 Sample was dispensed onto these nanowire grids using a picoliter piezo dispensing head. A total of ~5 nl sample
754 was dispensed in a stripe across each grid, followed by a pause of a few milliseconds, before the grid was plunged
755 into liquid ethane.

756 Data were acquired using the Leginon system installed on Titan Krios electron microscopes operating at
757 300kV and fitted with Gatan K2 Summit direct detection device. The dose was fractionated over 50 raw frames and
758 collected over a 10 s exposure time. Individual frames were aligned and dose-weighted.

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759 CTF was estimated using the GCTF package[71]. Particles were picked using DoG Picker within the Appion
760 pipeline[72]. 2D and 3D classifications were performed using RELION[73]. A map of unliganded BG505 SOSIP.664
761 (EMDB ID 5782), low-pass filtered to 60 Å was used as the starting point of 3D classification followed by 3D
762 refinement in either RELION or cryoSparc[74]. For the vFP16.02 and vFP20.01 complexes, after 3D classification in
763 RELION, an additional step of *ab initio* reconstruction was performed using cryoSparc.
764 **Cryo-EM Model fitting.** Fits of HIV-1 trimer and Fab to the cryo-EM reconstructed maps were performed using
765 Chimera[75]. Glycosylated BG505 SOSIP trimer structure (PDB ID: 5YFL) was used for the trimer fits. For antibody
766 fitting, we used the fusion peptide-bound coordinates of vFP1.01 and vFP5.01. For the antibody Fabs, both
767 orientations rotated ~180° about the Fab longitudinal axis were tested, and the optimal fit was decided based on map-
768 to-model correlation and positioning of the fusion peptide bound to the Fab relative to Env. For the vFP16.02 and
769 vFP20.01 bound complexes, the coordinates were further fit to the electron density by an iterative process of manual
770 fitting using Coot[68] and real space refinement within Phenix[69]. Molprobity[76] and EMRinger[77] were used to check
771 geometry and evaluate structures at each iteration step. Figures were generated in UCSF Chimera and PyMOL.
772 Map-fitting cross correlations were calculated using Fit-in-Map feature in UCSF Chimera. Map-to-model FSC
773 curves were generated using EMAN2. Local resolution of cryo-EM maps was determined using RELION.
774 **Defining vFP1 class antibody V-gene sequence signature.** The V-gene sequence signature for vFP1 class
775 antibodies were defined by examining the vFP1 class antibody sequences that neutralize at least seven out of the ten
776 tested isolates and the structures of FP in complex with vFP1.01, vFP16.02, and vFP20.01. A residue position was
777 considered as part of the sequence signature if at least one side chain heavy atom was within five angstroms from
778 any fusion peptide heavy atom for all three complex structures, and no more than three similar amino acid types
779 have a combined prevalence of more than 90%, and each of these amino acid types had a prevalence of more than
780 10%.
781 **Molecular dynamics simulation of mannose-5 Env trimer.** By using the BG505 SOSIP.664 Env trimer structure
782 (PDB ID: 4TVP) as a starting template, we modeled in a fully extended mannose 5 moiety at each *N*-linked
783 glycosylation sequon. The fusion peptide structure was then grafted onto our full mannose 5 model followed by
784 5000 steps of conjugate gradient energy minimization in implicit solvent using NAMD. The obtained structure was
785 then solvated in a 17 Å padding water box, neutralized by the addition of NaCl at a concentration of 150 mM. The

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786 CHARMM force field (https://www.charmm.org) was used for the parameterization of the protein (including CMAP
787 corrections and the mannose 9). TIP3P water parameterization[78] was used to describe the water molecules.
788 Two independent molecular simulation were carried out using ACEMD molecular dynamics software on their
789 METROCUBO workstation (https://www.acellera.com/products/GPU-hardware-molecular-dynamics-metrocubo/).
790 The system was minimized for 2000 steps, followed by equilibration using the NPT ensemble for 50 ns at 1 atm and
791 300 K using a time-step of 2 fs. We also used rigid bonds and a 9 Å cutoff using PME for long range electrostatics.
792 During the equilibration phase, heavy atoms on the protein were constrained by a 1 kcal/molÅ-2 spring constant and
793 slowly relaxed over the first 5 ns. Following the relaxation phase, the protein was allowed to move freely and
794 simulated for 500 ns under the NVT ensemble using ACEMD's NVT ensemble with a Langevin thermostat. To
795 achieve a time-step of 4 ps, we used damping at 0.1 ps-1 and a hydrogen mass repartitioning scheme. Each
796 simulation ran up to 500 ns.
797 The conformations of the fusion peptide (residue 512-519) were extracted from the MD simulations every 100
798 ps, producing an ensemble of 30'000 structures. Prody (http://prody.csb.pitt.edu) was used to perform the principal
799 component analysis of backbone atoms. The conformations of five crystalized fusion peptides were then projected
800 into the eigenspace defined by the first two components: vFP1.01, vF5.01, PGT151 (PDB: 5FUU), VRC34.01
801 (PDB: 5I8H) and clade G (PDB: 5FYJ).
802 **Analysis of antibody angle of approach to HIV-1 Env.** To compare modes of antibody recognition of HIV-1 Env
803 by vaccine elicited fusion peptide antibodies and VRC34.01, structural models of antibody in complex with HIV-1
804 BG505 SOSIP Env trimer derived from x-ray crystallography and EM were superimposed by pairwise alignment of
805 the Cα atom coordinates of the HIV-1 Env. To simplify the comparison, we first defined two common axes on the
806 HIV-1 Env, the trimer axis and the protomer axis, as reference lines. The trimer axis was defined by two points,
807 each with x, y, z coordinates obtained by averaging the coordinates of the Cα atom of a residue and its 3-fold
808 symmetry mates on the same trimer. The protomer axis was defined by a line perpendicular to the trimer axis that
809 passes the center of the protomer. On the antibody side, we also defined two axes for each Fab. The long axis of a
810 Fab was defined by two points, one point from the variable domain with x, y, z coordinates obtained by averaging
811 the coordinates of the Cα atom of the 4 conserved Cys (Cys 22 and Cys92 of heavy chain, and Cys23 and Cys88 of
812 light chain), and the other from the constant domain with x, y, z coordinates obtained by averaging the coordinates
813 of the Cα atom of the 4 conserved Cys (Cys 140 and Cys196 of heavy chain, and Cys134 and Cys194 of light

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814 chain). The short axis of a Fab was defined by a line connecting Cα atoms of heavy chain Cys22 and light chain
815 Cys23. Approaching angles of each antibody were then calculated as 1) angle between trimer axis and Fab long axis,
816 and 2) angle between the axis of major interaction protomer and Fab long axis. In addition, the relative heavy and
817 light chain orientation of antibody variable domains was compared by angles between the Fab short axes. The axes
818 were visualized in PyMOL by placing their coordinates in PDB format.
819 **Autoreactivity assay.** Antibodies were assessed for autoreactivity by testing for binding to HEp2 cells by indirect
820 immunofluorescence (Zeus Scientific, ANA HEp2 test system) and cardiolipin by ELISA (Inova Diagnostics,
821 QUANTA Lite ACA IgG III), per the manufacturer's instructions. On HEp2 cells, antibodies were assigned a score
822 between 0 and 3+ using control antibodies as reference. In the cardiolipin binding assay, OD values were converted
823 to GPLs using standard samples provided in the kit. Monoclonal antibodies that scored greater than 20 GPLs at 33
824 μg/ml were considered autoreactive.
825 **Neutralization fingerprinting analysis.** The neutralization fingerprint of a monoclonal antibody or polyclonal
826 plasma is defined as the potency pattern with which the antibody/plasma neutralizes a set of diverse viral strains.
827 The neutralization fingerprints of a set of monoclonal antibodies and NHP plasma were compared and clustered
828 according to fingerprint similarity, as described previously[79]. A set of 132 strains[80] was used in the neutralization
829 fingerprint analysis for Fig. S11, and the 58 FP-selected strains for Fig. 6.
830 **Associations between glycosylation patterns versus neutralization**. We calculated the associations between the
831 sequence variability of FP neighboring glycosylation sites (HXB2 numbering 88, 241, 448 and 611) and the large
832 panel neutralization using an approach implemented with R package SeqFeatR (https://seqfeatr.zmb.uni-due.de).
833 Briefly, for each residue position we create contingency table based on glycan occurrence and neutralization
834 sensitivity and evaluate the association using Fisher's exact test. The resulting P-values were corrected using
835 multiple testing method Holm.
836 **Statistical analysis**. The correlation between neutralization breadth on 58-isolate panel with FP8=AVGIGAVF and
837 neutralization breadth on the full 208-isolate panel was performed using Pearson correlation. The differences of
838 number of neutralized ioslates between FP-KLH primed+DS-SOSIP boosted group and DS-SOSIP alone group
839 were evaluated using one-tailed Mann-Whitney test. The differences of neutralization betwewen FP-KLH groups
840 and KLH-only group were evaluated using one-tailed Mann-Whitney test. The correlation between antibody trimer
841 binding and antibody neutralization breadth was performed using Pearson correlation. The differences of divergence

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842 of germline between antibodies from trimer-primed groups and antibodies from non-trimer-primed groups were
843 evaluated using two-tailed Mann-Whtiney test. The differences of sera neutralization between trimer-boosted
844 animals and non-trimer-boosted animals were evaluated using one-tailed Mann-Whitney test. The association of FP-
845 proximal N-glycan sequons (N88, N241, N448, and N611) and neutralization based on the 208-isolate panel was
846 evaluated using two-tailed Fisher's exact test. Unless otherwise indicated, all data were plotted and graphed using
847 GraphPad Prism, version 7.0. $P < 0.05$ indicated a statistically significant difference.
848 **Code availability**. No studies deemed central to the conclusions were carried out with custom code.
849 **Data availability**. All data generated or analyzed during this study are included in this published article (and its
850 Supplementary Information files). Atomic coordinates and structure factors of the reported crystal structures were
851 deposited in the Protein Data Bank with PDB ID 5TKJ, 5TKK, 6CDM, 6CDO, 6CDP. Cryo-EM reconstructions
852 were deposited in the Electron Microscopy Data Bank with EMDB accession code EMD-7460, EMD-7459, EMD-
853 8420, EMD-8421 and EMD-8422, and in Protein Data Bank with PDB ID 6CDI and 6CDE. Heavy chain- and light
854 chain-variable sequences of monoclonal antibodies vFP1.01 - vFP7.05 and vFP7.06 - vFP 32.07 were deposited with
855 GenBank under accession numbers KX949064 - KX949087 and MH017667 - MH017826, respectively.
856 **Life Sciences Reporting Summary**. Further information on experimental design is available in the Life Sciences
857 Reporting Summary.
858
859
860 **METHOD REFERENCES**
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905

906

907 **FIGURE LEGENDS**

908

909 **Figure 1** Design, properties, and immunogenicity of FP immunogens based on the epitope of
910 antibody VRC34.01. (**a**) Structure-based design, antigenic characteristics, and EM structure of
911 FP immunogens. The glycosylated structure of the HIV-1 Env trimer is shown at far left, with
912 exposed N-terminus of FP highlighted in red. Subsequent images show site recognized by
913 VRC34.01 antibody, schematics and antigenicity of FP immunogens, and negative stain EM of
914 FP-KLH (see Supplementary Fig. 1 for details of FP antigenicity). For EM study, n=3

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experiments were performed independent with similar results. (**b**) Immunization regimen 1. At day 52, mouse spleens were harvested and hybridomas created. (**c**) ELISA and neutralization of serum from regimen 1-immunized mice. Protein probes used for ELISA are defined in top row and include BG505 SOSIP.664 (green), FP-epitope scaffold based on PDB 1M6T (red), and 1M6T scaffold with no FP (blue). Column 1 defines mouse identification number and subsequent columns show ELISA and neutralization. ELISAs are shown as a function of serum dilution for pre-bleed, days 21, 35, and 52 (ELISA curves colored according to probe, with sera mostly unreactive with IM6T scaffold with no FP). Neutralization (ID_{50}, ID_{80}) values provided for day 52 serum; see Supplementary Fig. 2a for neutralization details. (**d**) Immunization regimen 2. At day 38, mouse spleens were harvested and hybridomas created. (**e**) ELISA and serum neutralization of serum from regimen 2-immunized mice, displayed as in **c**.

Figure 2 FP assumes disparate antibody-bound conformations, with neutralization restricted to a select angle of trimer approach. (**a**) Structural definition of vFP1.01 recognition. Top panels, cryo-EM reconstruction at 8.6 Å resolution (density shown in gray) of Fab vFP1.01 in complex with BG505 DS-SOSIP trimer. Expanded view, crystal structure of Env trimer and FP-bound Fab vFP1.01 at 2.0 Å resolution, as modeled into the cryo-EM map by rigid-body docking. Env trimer in green for gp120 and gray for gp41, Fab vFP1.01 in cyan for heavy chain and in yellow for light chain, and FP N-terminus in purple. Surface areas provided for N-terminal region of FP. (**b**) Same as **a**, but for vFP5.01 with FP in pink. Note that the angle of the lower right Fab differs from the angles of the other two. (**c**) Comparison of FP bound by vFP1.01 versus VRC34.01, with antibody shown in ribbons and FP in stick representation. (**d**) Same as **c**, but for vFP5.01. (**e**) Angle of recognition and Fv-domain overlap for vFP1.01, vFP5.01, and VRC34.01. Measured angles (red) are between antibody angle of approach and Env-trimer axis (left, with viral membrane located below trimer) and between antibody angle of approach parallel to viral membrane and Env protomer (right, looking down trimer axis towards viral membrane).

Figure 3 Second-generation vaccine-elicited antibodies neutralize up to 31% of HIV-1. Neutralization dendrograms display the diversity of tested viral strains, with branches colored according to neutralization potency (non-neutralized branches shown in gray). Top row: 208-strain panel for vaccine-elicited antibodies vFP16.02 and vFP20.01. Bottom left, 58-strain panel, displaying only branches with FP8=AVGIGAVF. Bottom right, comparison of breadth on 58- and 208-strain panels shown with Pearson correlation (n=7 antibodies).

Figure 4 Substantial glycan contacts by 2nd-generation FP-directed antibodies. (**a**) CryoEM map of quaternary complex with antibody 2712-vFP16.02, segmented by components at a contour level that allowed visualization of Fv domains of antibodies (also see Supplementary Figs. 9 and 10). (**b**) Same as **a**, but with 2716-vFP20.01. (**c**) Details of vFP16.02 interaction, with right panels showing experimental EM density in blue mesh, with contour level adjusted to allow visualizing of partially ordered glycan. Residues altered by SHM highlighted in cyan. Antibody vFP16.02 contacts both FP and neighboring glycans to achieve 31% breadth. (**d**) Same as **c**, but for vFP20.01. (**e**) Sequence alignment of vaccine-elicited FP-directed antibodies and origin genes. FP contacts (red highlight), glycan contacts (green rectangle) and SHM (cyan font) are highlighted. Additional Env contacts are indicated by double underlining. Because the density from the cryo-EM reconstructions was not always sufficient to allow for atomic-level fitting, contacts shown with dotted green rectangles were inferred.

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961
962 **Figure 5** Immunization of guinea pigs and NHPs with FP-coupled carriers and DS-SOSIP trimer
963 elicits heterologous neutralizing responses. (**a**) Elicitation of serum neutralizing responses in
964 guinea pigs. Immunization regimen and week 28 serum ID_{50} titers as measured on a 10-wildtype
965 strain panel, 5 with complete glycans around FP, and 5 naturally missing glycans at sites defined
966 in the figure. Also shown are titers for Δ88, Δ611, and Δ88+611 glycan-deleted variants of
967 BG505. (**b**) Plot comparing guinea pig-serum neutralization breadth for FP-KLH prime and DS-
968 SOSIP-trimer boost regimen versus DS-SOSIP alone regimen (one-tailed Mann-Whitney; see
969 Supplementary Fig. 7 for DS-SOSIP alone immunizations at 0, 4 and 16 weeks); n=5 animals for
970 each group. (**c**) Elicitation of heterologous neutralizing responses in rhesus macaques.
971 Immunization scheme, and week 46 serum titers assessed and displayed as in **a**. (**d**) Plot
972 comparing NHP-serum neutralization breadth for immunization regimens, displayed as described
973 in **b** for guinea pigs; n=5 animals for each group.
974
975 **Figure 6** Patterns of neutralization indicate FP-directed responses in mice and NHP are related.
976 (**a**) Neutralization on a 58-strain panel, comprising strains from the 208-strain panel with the FP
977 sequence, AVGIGAVF, matching both FP8-KLH and BG505 trimer immunogens. Top panel,
978 IC_{50} values for FP-directed antibodies. Bottom panel ID_{50} titers for NHP plasma: top rows, FP-
979 KLH prime and DS-SOSIP-trimer boost regimen at week 46, 2 weeks after third trimer boost;
980 bottom row, DS-SOSIP alone regimen at week 18, 2 weeks after third trimer boost. Number of
981 neutralized strains and neutralization breadths are shown. (IC_{80} and ID_{80} provided in
982 Supplementary Table 3c and Supplementary Fig. 7.) (**b**) Neutralization curves of NHP plasma
983 (DF1W week 46) and control monoclonal antibodies (VRC34.01 and VRC01) on four
984 representative strains in the presence of no peptide (black), FP (red) or an irrelevant Flag peptide
985 (blue). Mean and standard deviation of results from triplicated experiments shown (n=3
986 independent experiments). Location of representative tested strains are labeled and shown on
987 dendrogram in **c**. (**c**) Neutralization dendrogram (ID_{50}) for NHP DF1W week 46 plasma on 58-
988 strain panel. (**d**) Neutralization-fingerprint dendrogram calculated from 58-strain panel. Vaccine-
989 elicited vFP antibodies (highlight with green background) and three NHP week 46 plasma
990 (highlighted with forest green background for three of five NHP with sufficient neutralization to
991 yield accurate fingerprint analysis) clustered next to each other (see also fingerprint dendrogram
992 on 132-curated strains shown in Supplementary Fig. 11a).
993

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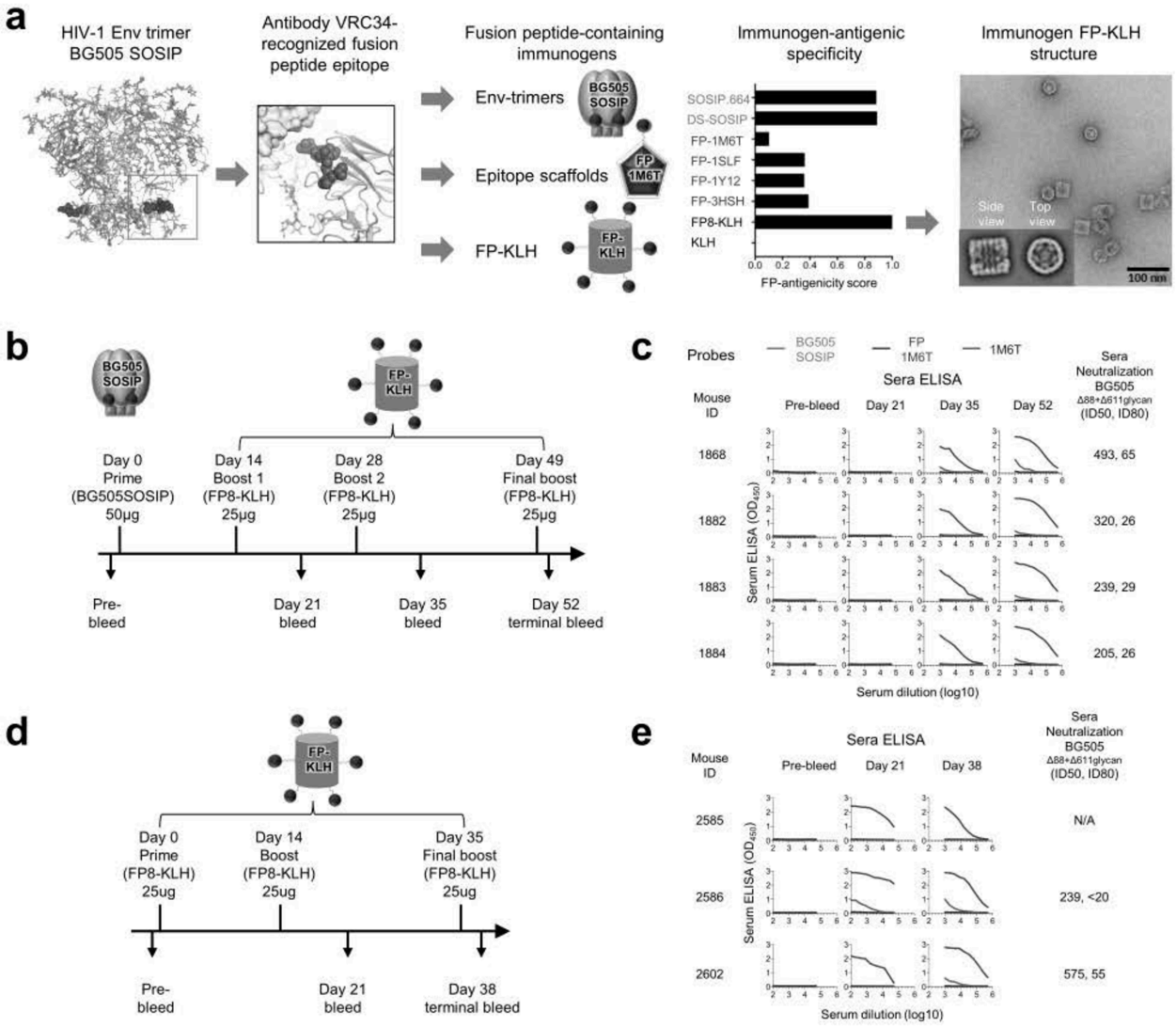

Figure 1

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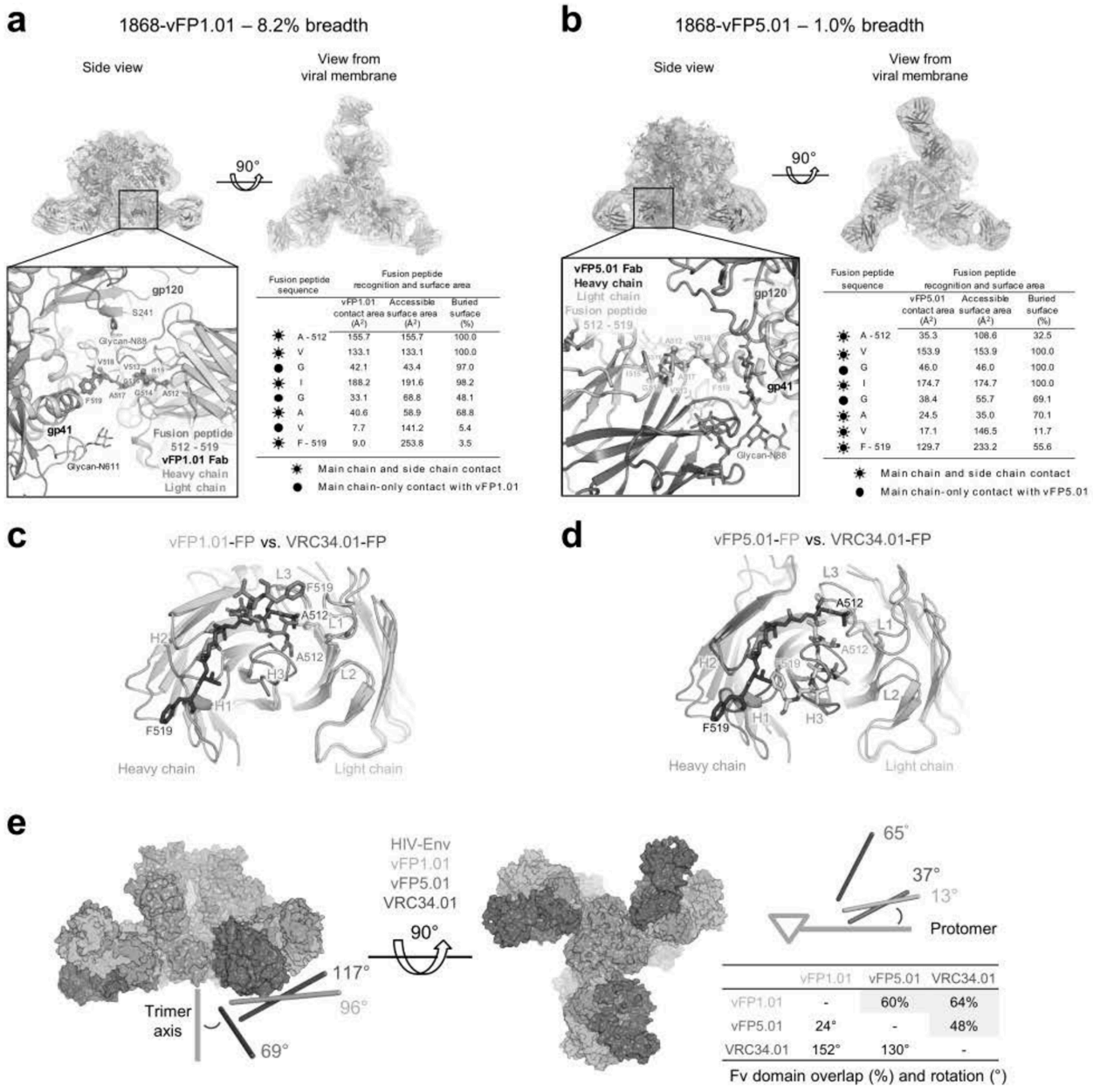

Fusion peptide sequence	Fusion peptide recognition and surface area: vFP1.01 contact area (Å²)	Accessible surface area (Å²)	Buried surface (%)
A - 512	155.7	155.7	100.0
V	133.1	133.1	100.0
G	42.1	43.4	97.0
I	188.2	191.6	98.2
G	33.1	68.8	48.1
A	40.6	58.9	68.8
V	7.7	141.2	5.4
F - 519	9.0	253.8	3.5

Fusion peptide sequence	Fusion peptide recognition and surface area: vFP5.01 contact area (Å²)	Accessible surface area (Å²)	Buried surface (%)
A - 512	35.3	108.6	32.5
V	153.9	153.9	100.0
G	46.0	46.0	100.0
I	174.7	174.7	100.0
G	38.4	55.7	69.1
A	24.5	35.0	70.1
V	17.1	146.5	11.7
F - 519	129.7	233.2	55.6

	vFP1.01	vFP5.01	VRC34.01
vFP1.01	-	60%	64%
vFP5.01	24°	-	48%
VRC34.01	152°	130°	-

Figure 2

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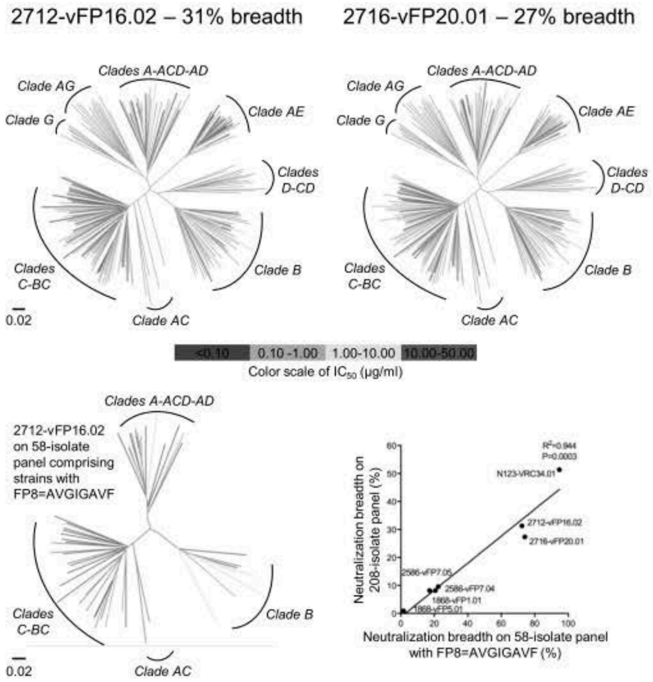

Figure 3

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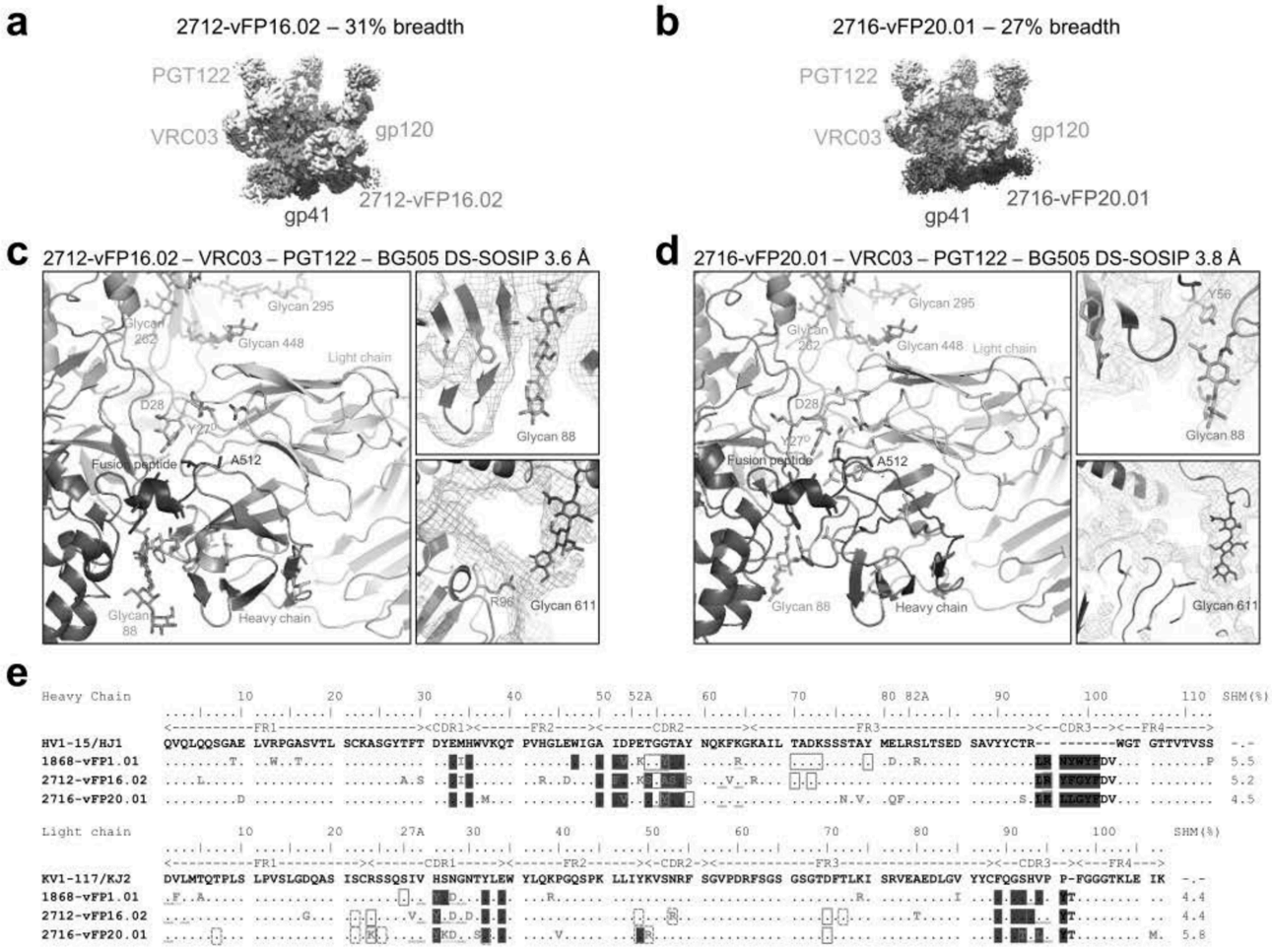

Figure 4

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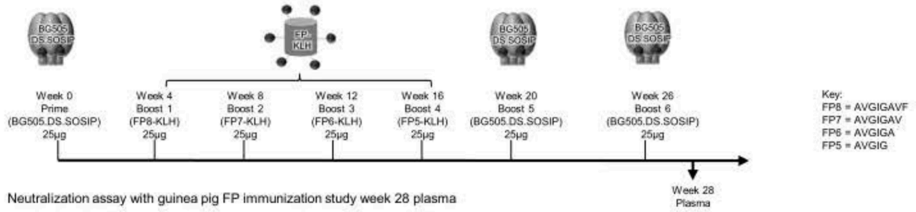
a Guinea pig FP immunization

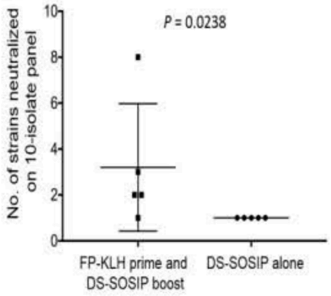


Neutralization assay with guinea pig FP immunization study week 28 plasma

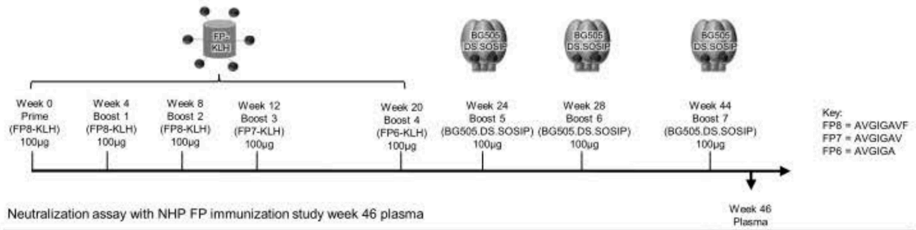
Clade	A		A		B		B		C		AE		BC		C		C		A		A		A		A	
Glycan missing	Complete		Complete		Complete		Complete		Complete		241+611		448		241		241		241		Δ88+241		241+Δ611		Δ88+241+Δ611	
Viruses	KER2008.12		Q23.17		3988.25		BL01.DG		286.36		CNE56		CNE19		25710-2.43		0077.V1.C16		BG505		BG505 Δ88		BG505 Δ611		BG505 Δ88+611	
Guinea pig #	ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80
CGP701-1	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	70	<20	<20	<20	45	<20	<20	<20	81	<20	145	22	6,149	953	29,545	8,365
CGP701-2	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	2,085	389	1,267	257	6,857	999	10,698	870
CGP701-3	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	58	<20	<20	<20	72	<20	<20	<20	161	71	226	46	3,406	1,062	24,920	6,195
CGP701-4	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	33	<20	<20	<20	36	<20	23	<20	2,295	534	13,345	2,922
CGP701-5	161	<20	151	<20	128	<20	148	<20	<20	<20	141	<20	275	37	496	104	<20	<20	106	25	433	104	4,997	1,471	17,286	5,416

<20 | 20-100 | 100-1000 | 1000-10000 | >10000

b



c NHP FP immunization



Neutralization assay with NHP FP immunization study week 46 plasma

Clade	A		A		B		B		C		AE		BC		C		C		A		A		A		A	
Glycan missing	Complete		Complete		Complete		Complete		Complete		241+611		448		241		241		241		Δ88+241		241+Δ611		Δ88+241+Δ611	
Viruses	KER2008.12		Q23.17		3988.25		BL01.DG		286.36		CNE56		CNE19		25710-2.43		0077.V1.C16		BG505		BG505 Δ88		BG505 Δ611		BG505 Δ88+611	
NHP #	ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80
DF3R	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	113	<20	167	<20	172	25	604	70
DFPE	<20	<20	31	<20	<20	<20	<20	<20	<20	<20	<20	<20	85	20	104	28	<20	<20	180	23	231	28	806	196	2,365	500
DF1W	24	<20	145	20	<20	<20	91	24	103	23	<20	<20	33	<20	741	170	98	<20	205	<20	450	60	6,131	1,571	29,016	3,998
DF4B	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	26	<20	63	<20	<20	<20	22	<20	38	<20	913	187	4,772	658
0PV	<20	<20	29	<20	<20	<20	<20	<20	<20	<20	<20	<20	28	<20	201	49	<20	<20	66	<20	77	<20	1,231	292	5,211	1,386

<20 | 20-100 | 100-1000 | 1000-10000 | >10000

d

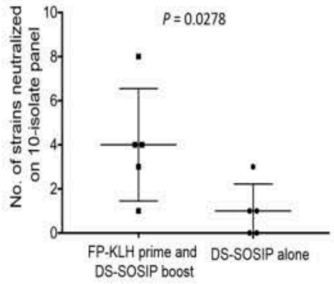


Figure 5

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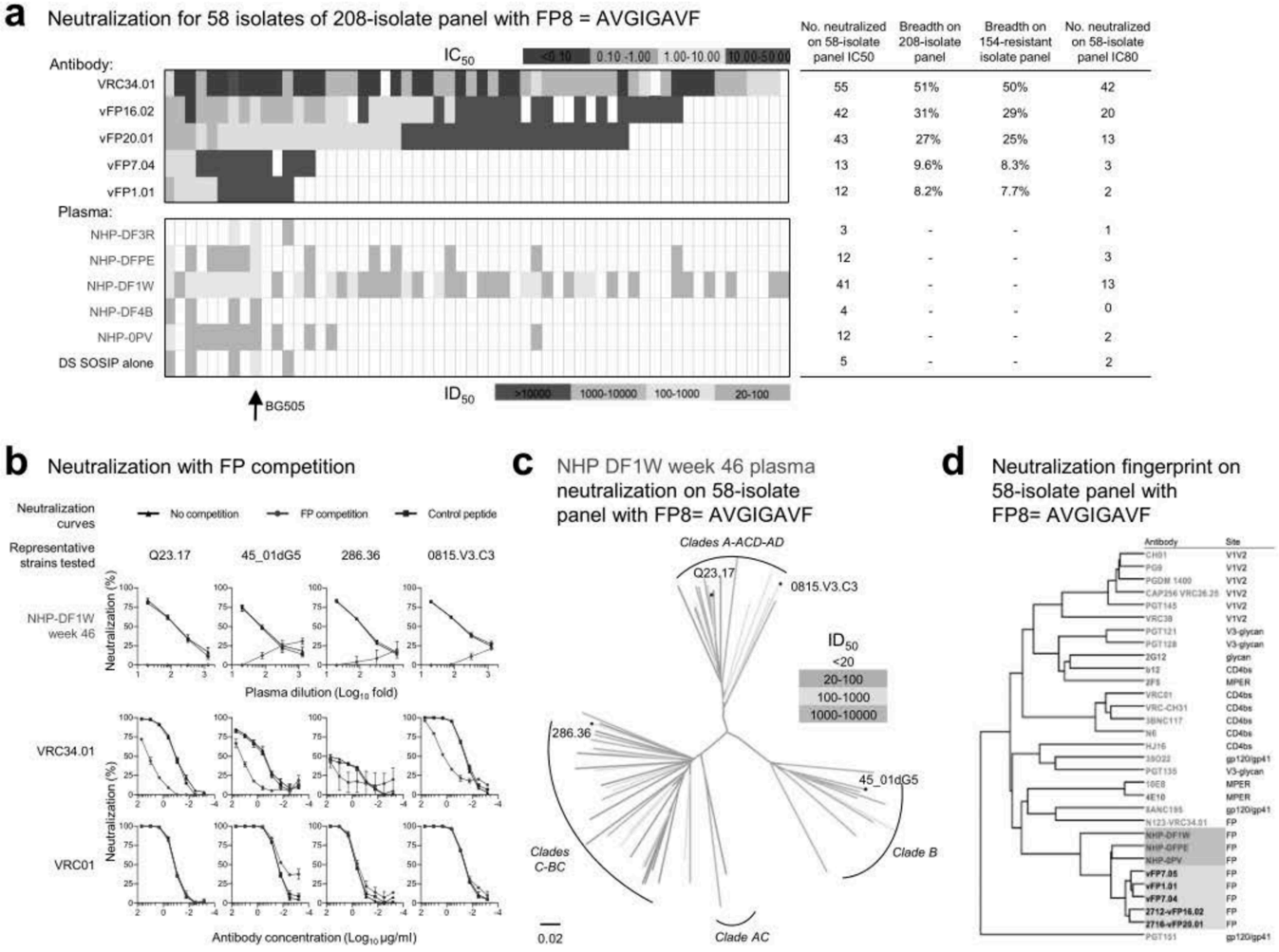

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Figure 6

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Thu, 3 May 2018 15:18:27 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Vance, Meaghan (NIH/NIAID) [C];Folkers, Greg (NIH/NIAID) [E]
Subject: FW: Event Logistics and Schedule - May 9, 2018
Attachments: Lazarus Effect Engage Outline 5 3 18.docx

Please see attached. The prep call is Friday – please advise if you need anything for this prep call and/or the event on the 9th.

Meaghan – please add copy to folder.

Patricia L. Conrad
Special Assistant to the Director

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From: Walker, Annie (b)(6)
Sent: Thursday, May 03, 2018 11:14 AM
To: (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Prince, Emma (b)(6) Sullivan, Kevin (b)(6)
Subject: RE: Event Logistics and Schedule - May 9, 2018

Mark and Dr. Fauci,
I have you both confirmed to brief with Sully tomorrow at 3:00 p.m. CST. Please review the attached document in advance of the call.

Annie Walker
Event Assistant
George W. Bush Presidential Center
T: (b)(6) | M:
E: (b)(6)

From: Walker, Annie
Sent: Wednesday, May 02, 2018 10:43 AM
Cc: Prince, Emma (b)(6)
Subject: Event Logistics and Schedule - May 9, 2018

Panelists,

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Thank you so much for joining us next Thursday, May 9 for our special ENGAGE Event: *The Lazarus Effect, 15 Years Later.* Attached is a speaker schedule for your reference. Could you please confirm your attendance for the dinner following the Engage program on May 9? Also, please let us know if you will have a guest, and if there are any dietary restrictions we need to be aware of for the evening. The dinner will be hosted by President and Mrs. Bush and take place in our Presidential Reception Hall immediately following the event.

Also, I have confirmed transportation to and from the hotel to the event, noted in the attached speaker schedule. If you need transportation to and from the airport, please let me know as soon as possible so I can coordinate. You are also welcome to Uber and submit your receipts to me for reimbursement.

Thank you in advance! We are looking forward to next week.

Best,
Annie Walker

GEORGE W. BUSH
PRESIDENTIAL CENTER

ANNIE WALKER
EVENT ASSISTANT

GEORGE W. BUSH PRESIDENTIAL CENTER
T: (b)(6) | E: (b)(6)

WWW.BUSHCENTER.ORG

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May 3, 2018

The Lazarus Effect: 15 Years Later // Overview

May 2018 is the 15th anniversary of the President's Emergency Plan for AIDS Relief (PEPFAR), which was signed into law by President Bush. PEPFAR is credited with saving millions of lives and today supports more than 13.3 million people with lifesaving antiretroviral treatment. As President Bush wrote in his memoir, *Decision Points*, "People who were given up for dead were restored to healthy and productive lives…Africans came up with a phrase to describe the transformation. They called it the Lazarus Effect."

To celebrate the life-changing success of PEPFAR and other related programs made possible by the generosity of the American people, **Barbara Bush**, co-founder of Global Health Corps, will moderate two panels on the impact and future of global health involvement.

Panel 1: The largest international health initiative in history for a single disease/saving lives and changing the paradigm. Participants:

- Former Rwandan Minister of Health **Dr. Agnes Binagwaho**
- Key architect of PEPFAR **Ambassador Mark Dybul, M.D.**
- *Washington Post* columnist **Michael Gerson**

<u>Key topics</u>:

- Stories from those who were present at the birth and implementation of PEPFAR
- Themes to draw out:
  - There was bold, bipartisan leadership
  - Compassion and generosity of the American people
  - Powerful survivor stories (is there a surivivor we could have in the room?)
  - PEPFAR as the manifestation of a transformative, new paradigm - The Monterrey Consensus: moving from paternalism to partnership and service/from money spent to results achieved and good governance/from government to government to people to people, all sectors need to engage including private sector.
  - Driving entire US government approach based on data
  - First ever national scale, chronic prevention and care – opening door to all chronic disease.

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Panel 2: The state of health in Africa today…and looking forward, getting to the end of the epidemic. Participants:

- Key architect of PEPFAR **Dr. Anthony Fauci**
- Partner in Health's **Dr. Paul Farmer**
- **Favorite "Favo" Iradukunda**, former Global Health Corp Fellow from Rwanda
- **Senator Lindsey Graham** (R-South Carolina)

Key topics:

- Scientific innovation coming, we need it to get to the end
- Need to stay engaged/U.S. government leadership and funding
- Focus on adolescents and young people/girls and boys – could lose control of the epidemic that requires a new approach beyond just medical intervention
- Using the platform for person-centered care/PRRR, etc. (Note – we need to see where we are in the PRRR transition and adapt comments about PRRR)

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HHS-NIAID-20-0593-A-001099

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Thu, 3 May 2018 13:50:37 +0000
To: Paules, Catharine (NIH/NIAID) [E]
Subject: FW: Influenza hemagglutinin with lysozyme-like sequence motifs

Let us discuss.

From: Jen Lo (b)(6)
Sent: Thursday, May 03, 2018 8:36 AM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Challberg, Mark (NIH/NIAID) [E] (b)(6)
Subject: Re: Influenza hemagglutinin with lysozyme-like sequence motifs

Dear Dr. Fauci,

Good morning! Hope you find the lysozyme-related influenza hemagglutinin (HA) information in my email (04/20/18) useful and helpful to NIH in support of its effort to develop the "Universal" influenza vaccine. Finding these conserved lysozyme-like sequence motifs in influenza HA is significant especially when N-acetyl-glucosamine is a common component in their respective binding targets (i.e. Sialic acid receptor for flu virus and bacterial peptidoglycan for lysozyme). Thus, lysozyme-like influenza HA might interfere with the normal antimicrobial function of lysozyme in vivo and create greater opportunities for the occurrence of potentially deadly secondary bacterial infection in flu patients. Furthermore, the similarities in sequence motifs between endogenous lysozyme and HA might also impact the antigenicity/immunogenicity of the HA epitopes, affecting the effectiveness of flu vaccine comprising the whole HA protein.

I wish to work with the NIH researchers testing the vaccine feasibility of some of the never-before-known lysozyme-like HA peptides as proposed in my previous email (these peptides can be made available to NIH researchers for testing in animals if needed) and look forward to your favorable reply soon.

Best regards,
Jennifer

Jennifer Lo, PhD
CEO & President
BioJENC

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Thanks
> On Apr 20, 2018, at 3:49 AM, Jen Lo (b)(6) wrote:
>
> Dear Dr. Fauci,
>
> I am writing this letter to share for the first time my recent finding concerning influenza vaccine with NIH. Although influenza has been the subject of many investigations since the historical 1918 influenza pandemic, there have been very few reports on influenza association with lysozyme. My recent finding on the significant sequence similarity shared between lysozyme and hemagglutinin of influenza virus might help better our understanding of the pathogenesis of influenza virus, human transmissibility, and the immunity (or the lack of) against influenza infection, and may provide opportunities for a new/different approach to help develop an effective "Universal" subtype flu vaccine.
>
> As you know, lysozyme is abundant in different components (e.g. phagocytes, tears, saliva, mucus) of the body. Any proteins (and peptides) with an amino acid sequence similar to lysozyme may act as lysozyme inhibitors (e.g. Interfering with the substrate binding). Lysozyme plays an important role in our immune response, especially when it is expressed by neutrophils to help digest ingested microbes (neutrophils are regarded as the first responders in an immune response to infection and inflammation). Neutrophils may also act as antigen presenting cell and their multifunction is already at work when symptoms emerged. Thus, the presence of abundant potential lysozyme inhibitors (i.e. Influenza hemagglutinin/influenza virus) due to influenza infection may adversely affect the intended functions of immune neutrophils, effecting the ability of the host to:
> 1. induce a desired humoral and cell mediated immunity against influenza viruses,
> 2. avoid a strong inflammatory response and prevent an undesired occurrence of cytokine storm,
> 3. prevent/fight secondary bacterial infection (a significant contributing factor to flu-related mortality and morbidity)
>
> The similar sequence motifs shared between lysozyme and hemagglutinins of different subtypes of influenza virus are shown with highlights (different highlight colors are used to compare/contrast different motifs between H1, H3, H5 subtypes compared to human and chicken lysozyme) as follows (besides the sialic acid receptor factor, this lysozyme-like sequence motif might also play a contributing role in human transmissibility of a flu strain, where the greater the sequence identity, the stronger the lysozyme inhibition, the more efficient the infectivity, the easier the and the greater the risk of secondary bacterial infection):
>
>
>
> Inducing an effective immune response that targets against the influenza hemagglutinin (HA) has been preferred over the neuraminidase for HA's essential cell binding function, but the HA conserved lysozyme-like sequence motifs as shown herein indicate the potential challenge of using whole virus or whole HA to induce an effective neutralizing antibody response against these epitopes (in bold) of HA of Groups I & II influenza A viruses that would meet the 4 criteria stated in the new strategic plan for developing a "universal" flu vaccine. I think peptide vaccines might have a better chance to help achieve the goal.
>
> An ideal flu vaccine would induce a long lasting strong antibody/immune response that has the specificity against the conserved lysozyme-like sequence motif but has no cross reactivity against the

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endogenous Lysozyme (not known if the lysozyme peptides as shown herein might have any inhibitory effects on the influenza viruses).
>
> These shared sequence motifs might play an important role in the design and development of a human flu vaccine that will lead to an effective and long lasting "Universal" subtype flu vaccine for all age groups. I think an HA peptide with modified amino acid sequence based on their conserved lysozyme-like sequence motif may be used in development of an effective flu vaccine. As my resources are limited, I wish to work with Researchers at NIH on vaccine feasibility testing of these important flu peptides comprising the lysozyme-like sequence motifs.
>
> Thanking you in advance for your consideration and approval of my proposed vaccine feasibility testing on newly found lysozyme-like Influenza HA peptides.
>
> Sincerely,
> Jennifer
>
> Jennifer Lo, PhD
> CEO & President
> BioJENC
>
> Confidentiality Notice: This e-mail transmission may contain confidential or legally privileged information of BioJENC that is intended only for the individual or entity named in the e-mail address. If you are not the intended recipient, you are hereby notified that any disclosure, copying, distribution, or reliance upon the contents of this e-mail is strictly prohibited. If you receive this e-mail transmission in error, please reply to the sender so that proper delivery can be arranged, then please delete the message from your inbox. Thank you. Copyright 2018 BioJENC LLC. All rights reserved.
>

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Thu, 3 May 2018 13:49:50 +0000
To: Conrad, Patricia (NIH/NIAID) [E]
Subject: RE: factcheck-mgh-proto-flu-niaid

My quotes look fine. Thanks.

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Thursday, May 03, 2018 8:34 AM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: FW: factcheck-mgh-proto-flu-niaid

Can you please confirm or edit your quotes...tx

Patricia L. Conrad
Special Assistant to the Director

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From: charles kotsonis (b)(6)
Sent: Wednesday, May 02, 2018 9:29 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6) Taubenberger, Jeffery (NIH/NIAID) [E] (b)(6) Mascola, John (NIH/VRC) [E] (b)(6)
Subject: factcheck-mgh-proto-flu-niaid

Attn: Ms. Patricia Conrad

NIAID

Dear Anthony S. Fauci, MD, John R. Mascola, MD, and Jeffery K. Taubenberger, MD, PhD,

I am fact checking an article by Tim Gower for Massachusetts General Hospital's *Proto* magazine.

Kindly confirm the accuracy of your respective quotes in the attached doc, and let me know if any changes are required.

Thank you very much for your time and attention,

Charles Kotsonis
factsMaam.com

Researcher for Time Inc. Content Solutions / Meredith
(b)(6)
(b)(6)

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Thu, 3 May 2018 12:34:21 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: FW: factcheck-mgh-proto-flu-niaid
Attachments: factcheck-mgh-proto-flu-niaid.docx

Can you please confirm or edit your quotes...tx

Patricia L. Conrad
Special Assistant to the Director

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From: charles kotsonis (b)(6)
Sent: Wednesday, May 02, 2018 9:29 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6) Taubenberger, Jeffery (NIH/NIAID) [E] (b)(6) Mascola, John (NIH/VRC) [E] (b)(6)
Subject: factcheck-mgh-proto-flu-niaid

Attn: Ms. Patricia Conrad

NIAID

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Kindly confirm the accuracy of your respective quotes in the attached doc, and let me know if any changes are required.

Thank you very much for your time and attention,

Charles Kotsonis
factsMaam.com

Researcher for Time Inc. Content Solutions / Meredith
(b)(6)
(b)(6)

ProtoMag.com
DISPATCHES FROM THE FRONTIERS OF MEDICINE

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factcheck-mgh-protomag-flu-niaid – Fauci, Mascola & Taubenberger

TEXT:

{CONTEXT: The influenza virus causes an epidemic in the United States every year, year after year. Sometimes its passage is mild, with relatively few infections and low mortality. Other years it leaves a deeper mark, as it did last winter, with news of overflowing intensive-care units and children who fell ill one day and perished the next. While infectious disease experts and others who study the flu are never certain just how bad a season will be before it hits, they say that one thing is easier to predict: Some day, sooner or later, the world will face another serious pandemic — one that evokes the tragedy that struck in 1918…}

A microbe that carries the pathogenic punch of the 1918 influenza could still prove devastating, says virologist **Jeffery K. Taubenberger** of the **National Institute of Allergy and Infectious Diseases (NIAID)**, especially for people around the globe who lack access to advanced medical care.

"Many would not be much better off than in 1918," says Taubenberger, who believes another death toll of 100 million would not be out of the question.

{Discussion of approaches to influenza mutation and research toward a universal vaccine…}

Some groups are trying new ways to make vaccines with headless hemagglutinin.

A team led by scientists at NIAID has made headless stalks more stable by anchoring them to the blood protein ferritin. With such a vaccine, the body detects the presence of hemagglutinin, and the immune system responds as it would to a real virus, says **John Mascola**, director of NIAID's **Vaccine Research Center**.

In a 2015 study published in *Nature Medicine*, Mascola and his colleagues reported that a vaccine made with headless hemagglutinin stalks protected mice and ferrets from a dose of H5N1 avian flu that was lethal to unvaccinated lab animals. [...]

{B cells in the immune system generate the antibodies that often block a flu virus's infection of a cell. But if antibodies fail, T cells confer another layer of protection, producing immune cells called cytokines that can enter an infected cell and stop influenza from proliferating. Moreover, immunity based on T cells remains high after recovery from the flu, reducing the risk of re-infection, even by other subtypes of the disease. There are limits to what T cells can do, however. The immunity they provide waxes and wanes, and declines over a lifetime, says Sarah Gilbert, a vaccinologist at the University of Oxford and cofounder of Vaccitech, a biotech firm in Oxford, England. BiondVax and Vaccitech both are developing vaccines designed to boost T cell response against influenza by teaching the immune system to recognize and target proteins inside the virus's core, which — unlike surface proteins that trigger production of antibodies —

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don't change from year to year. In one trial, BiondVax's vaccine, known as M-001, was given to elderly men and women before they received the traditional flu vaccine, improving their immune response. More intriguing, tests three years later on stored blood taken from these men and women showed that the vaccine also neutralized the H3N2 virus that caused widespread illness in the 2014-2015 flu season, suggesting that the injection might guard against viruses that evolve and circulate in the future. BiondVax is now preparing phase 3 clinical trials, while Vaccitech is testing its vaccine in phase 2 trials.}

Similarly, Taubenberger and his colleagues at NIAID have tested a vaccine cocktail containing hemagglutinins from four subtypes of influenza — H1 and H3, which are typically included in seasonal flu vaccines, and H5 and H7, from avian viruses that have also infected people.

This not only protected lab animals from those flu subtypes, but also H2 viruses, H6 viruses and other influenza subtypes that were lethal to other animals. How could it protect against types not included in the vaccine?

Taubenberger says that part of the explanation may be that their vaccine cocktail, which triggers production of protective antibodies, may also stimulate T cells.

Despite the promise of these disparate approaches, a truly universal influenza vaccine is likely decades away, says **Anthony Fauci**, director of NIAID and a leading voice in the call for better flu protection.

Still, Fauci believes that broadly effective vaccines that protect against more than one subtype of the flu could be available much sooner.

"There is going to be universal flu vaccine 1.0, then 2.0 and so on," says Fauci. "We're not going to get the right answer the first time."

Other researchers question whether there will ever be a single flu vaccine that can provide all or even most people with something approaching total immunity. One somewhat controversial theory holds that a person's immune system may be "imprinted" by the first flu virus it encounters and then will primarily produce antibodies to that virus subtype, paying less attention to others.

That hypothesis isn't universally accepted, but if true, the immune systems of older people might be too rigid to respond to a universal vaccine. Very young people, in contrast, who had never been exposed to the flu, would have immunological clean slates and could be strong candidates.

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"You could produce generations of people who would be pretty resistant to new flu strains and new pandemics," says Taubenberger, "but it might not help fifty-somethings like me." [...]

Emerging technology could also speed production of vaccines in the event of a pandemic.

For example, DNA vaccines currently under investigation could be ready within a few months after the genome of a pandemic virus is sequenced, says Mascola.

The vaccines that Mascola and his colleagues have tested contain only the genetic sequence for hemagglutinin, minus the virus's protein coat.

"Muscle cells take up the DNA and make part of the flu virus — just the hemagglutinin — and the body produces an immune response," says Mascola.

In a 2017 study by NIAID scientists, 30 [28?] healthy adults were "primed" with a DNA vaccine, then received a second vaccine against the H7N9 virus, which is believed to have pandemic potential.

In the study, 78% to 100% of participants who received the primer DNA vaccine [IS THIS CORRECTLY PHASED?] had at least a four-fold increase in antibodies against the H7N9 virus.

Creating better flu vaccines will require not only patience, but a knowledge of history, says Taubenberger.

It would be a mistake to believe that the terrifying virulence of the 1918 pandemic was an outlier caused by strange mutations in the virus that are unlikely to recur.

"There are other viruses out there in the wild that share those features," says Taubenberger. "Something like this could happen again."

END TEXT.

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Thu, 3 May 2018 11:32:08 +0000
To: Fauci, Anthony (NIH/NIAID) [E];Marston, Hilary (NIH/NIAID) [E]
Subject: Discuss IAS keynote draft with Hilary

Does ASF have time Thursday or Friday to go over the current IAS keynote draft? If not, early next week?

Thanks,
Hilary

Hilary Marston, MD, MPH
Medical Officer and Policy Advisor for Global Health
NIAID Office of the Director
(b)(6)
(b)(6) (cell)

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Thu, 3 May 2018 01:08:03 +0000
To: Paules, Catharine (NIH/NIAID) [E]
Subject: FW: Please disregard previous figs - Revised fig attached for entire US & in terms of savings
Attachments: Annual Projected Benefits of a Universal Flu Vaccine.pdf, Annual Projected Benefits of a Universal Flu Vaccine.svg

fyi

From: Alison Galvani (b)(6)
Sent: Wednesday, May 02, 2018 8:53 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Sah, Pratha (b)(6) Meagan Fitzpatrick (b)(6) Paules, Catharine (NIH/NIAID) [E] (b)(6)
Subject: Re: Please disregard previous figs - Revised fig attached for entire US & in terms of savings

If it is not too late, the attached figure with revised y-axis label and title should be even easier to understand.

Your talk yesterday was as inspiring as always!

On Wed, May 2, 2018 at 5:44 PM, Fauci, Anthony (NIH/NIAID) [E] (b)(6) wrote:
Thanks, Alison. This is very helpful.

From: Alison Galvani (b)(6)
Sent: Tuesday, May 01, 2018 12:56 AM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Sah, Pratha (b)(6) Meagan Fitzpatrick (b)(6)
Subject: Please disregard previous figs - Revised fig attached for entire US & in terms of savings

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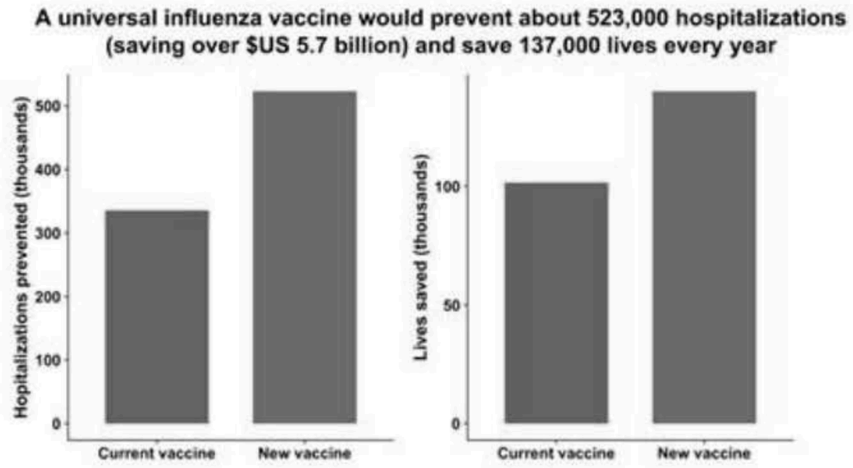

New figure for the entire US. This figure includes economic benefits and presents results in terms of savings (rather than reduced losses). Now very simple, intuitive and positive.

--

Alison Galvani, PhD

Director, Yale Center for Infectious Disease Modeling and Analysis (CIDMA)
Burnett and Stender Families Professor of Epidemiology
Yale School of Public Health
Yale School of Medicine
New Haven, CT 06520

(b)(6)
(b)(6)

--

Alison Galvani, PhD

Director, Yale Center for Infectious Disease Modeling and Analysis (CIDMA)
Burnett and Stender Families Professor of Epidemiology
Yale School of Public Health
Yale School of Medicine
New Haven, CT 06520

203-785-2642
http://cidma.yale.edu/

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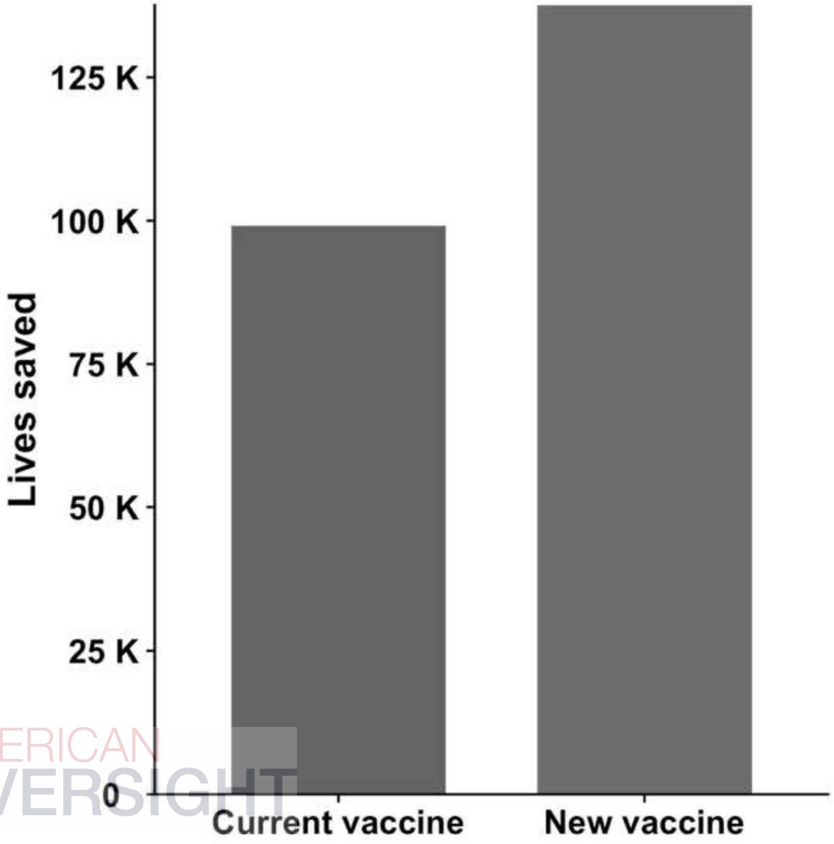
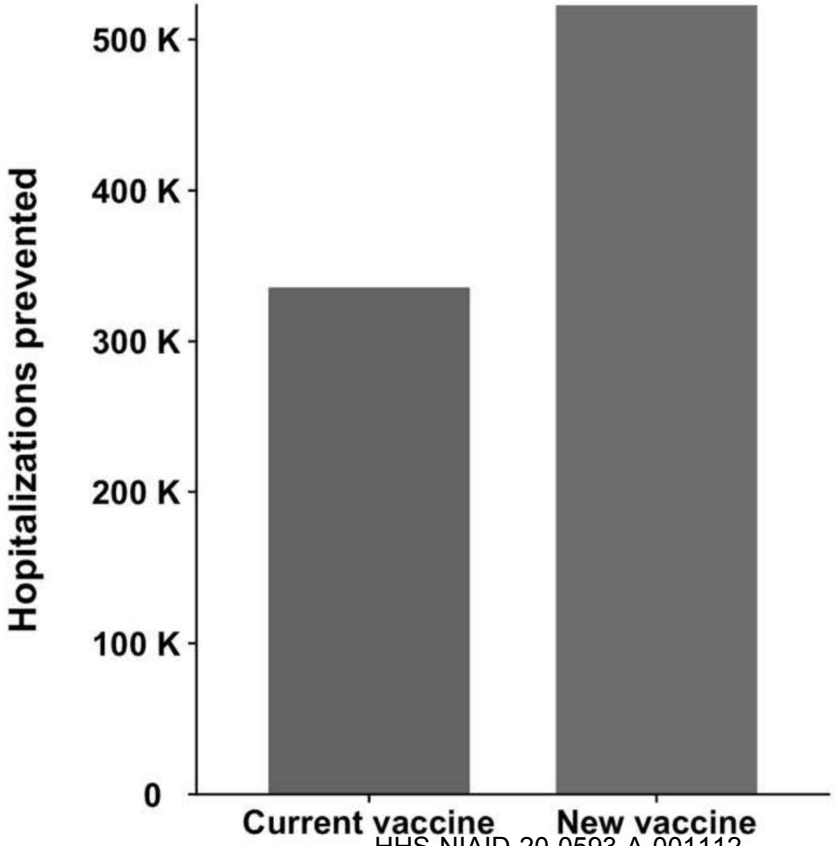
A universal influenza vaccine would save 137,000 lives, $5.7 billion and prevent 523,000 hospitalizations every year





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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Wed, 2 May 2018 18:22:53 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Harvard Law school - Outbreak Week Conference (keynote)
Attachments: Re: Invitation to speak at Harvard University , RE: Invitation to speak at Harvard University , Re: Invitation to speak at Harvard University

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From: Yansen, Shana Lynne
Sent: Wed, 2 May 2018 18:19:19 +0000
To: Conrad, Patricia (NIH/NIAID) [E]
Cc: Evans, Stacy L;Jonas, Olga B;Martin, Corrie
Subject: Re: Invitation to speak at Harvard University

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AMERICAN OVERSIGHT

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Patricia L. Conrad
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Shana

Shana L. Yansen, MHS | Program Manager
Harvard Global Health Institute
42 Church Street, Cambridge, MA 02138
o: (b)(6) email: (b)(6)
t: @harvardgh

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Sent: Thursday, April 19, 2018 4:23 PM
To: (b)(6)

AMERICAN OVERSIGHT

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Professor of Medicine, Harvard Medical School
http://ashishkjha.com |@ashishkjha

AMERICAN OVERSIGHT

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Sent: Fri, 8 Jun 2018 17:37:05 +0000
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Cc: Greg Folkers (b)(6) Meaghan (NIH/NIAID) [C]
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To: Conrad, Patricia (NIH/NIAID) [E]
Cc: Folkers, Greg (NIH/NIAID) [E];Vance, Meaghan (NIH/NIAID) [C]
Subject: Re: Invitation to speak at Harvard University

This is terrific news, Patty! Thank you. We are delighted he can join us.
Right now, we have him down for the keynote at 9:15am. The symposium is scheduled to start at 9am (breakfast and networking start at 8:30am).
Welcoming remarks will begin at 9am by Dr. Ashish K. Jha, Dean Michelle Williams and Prof. Glen Cohen, Director of the Petrie-Flom Center at HLS.
Depending on the flight schedules, we could potentially adjust that timing for Dr. Fauci. Just let me know.
I will get back to you early next week on desired length and Q&A.
Very much look forward to working with you on this as well!
Have a wonderful weekend, Patty and thanks again,
Shana
From: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Sent: Friday, June 8, 2018 1:37:06 PM
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AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Wed, 2 May 2018 21:56:58 +0000
To: Greg Folkers (b)(6)
Cc: Paules, Catharine (NIH/NIAID) [E]
Subject: FW: PNAS: Optimizing the impact of low-efficacy influenza vaccines

We should discuss this for a slide.

From: Folkers, Greg (NIH/NIAID) [E]
Sent: Wednesday, May 02, 2018 5:52 PM
Subject: PNAS: Optimizing the impact of low-efficacy influenza vaccines

Optimizing the impact of low-efficacy influenza vaccines

Pratha Sah, Jan Medlock, Meagan C. Fitzpatrick, Burton H. Singer and Alison P. Galvani
PNAS April 30, 2018. 201802479; published ahead of print April 30, 2018.
https://doi.org/10.1073/pnas.1802479115

1. Contributed by Burton H. Singer, March 30, 2018 (sent for review February 9, 2018; reviewed by Anthony S. Fauci and David Fisman)

- Article
- Figures & SI
- Authors & Info
- PDF

Significance

The efficacy of the influenza vaccine against the predominant influenza strain appears to be relatively low during this 2017–2018 season. Our analyses demonstrate the substantial effect of even low-efficacy vaccines in averting infections, hospitalizations, and particularly deaths. Our results also demonstrate that the health burden resulting from influenza is more sensitive to changes to vaccination coverage than to changes to vaccine efficacy. We further determined the uptake distribution of the 140 million doses available that would maximize the effectiveness of vaccination. Our results inform current public health policies and underscore the importance of influenza vaccination.

Abstract

The efficacy of influenza vaccines varies from one year to the next, with efficacy during the 2017–2018 season anticipated to be lower than usual. However, the impact of low-efficacy vaccines at the population level and their optimal age-specific distribution have yet to be ascertained. Applying an optimization algorithm to a mathematical model of influenza transmission and vaccination in the United States, we determined the optimal age-specific uptake of low-efficacy vaccine that would minimize incidence, hospitalization, mortality, and disability-adjusted life-years (DALYs), respectively. We found that even relatively low-efficacy influenza vaccines can be highly impactful, particularly when vaccine uptake is optimally distributed across age groups. As vaccine efficacy declines, the optimal distribution of vaccine uptake shifts toward the elderly to minimize mortality and DALYs. Health practitioner

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encouragement and concerted recruitment efforts are required to achieve optimal coverage among target age groups, thereby minimizing influenza morbidity and mortality for the population overall.

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From: Paules, Catharine (NIH/NIAID) [E]
Sent: Wed, 2 May 2018 21:56:06 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Re: Tomorrow AM
Attachments: image001.png

Thanks!

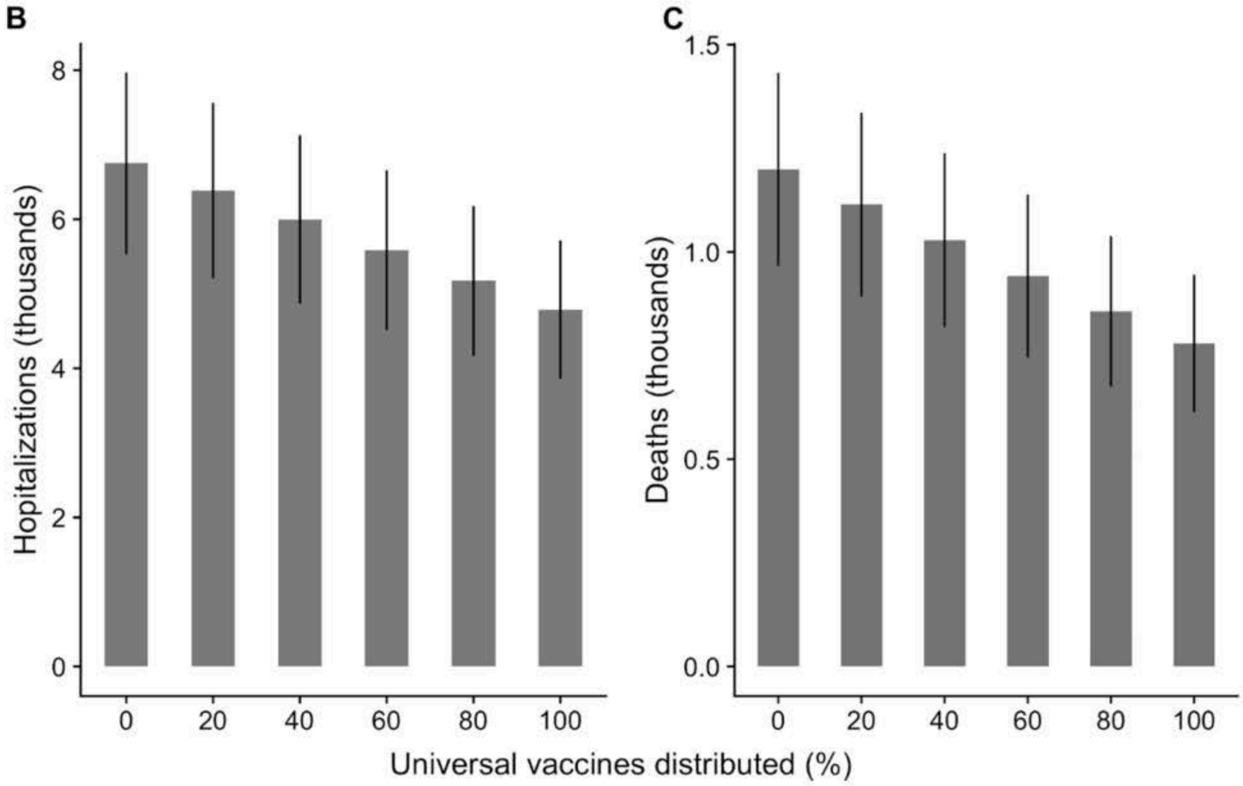
Sent from my iPhone

On May 2, 2018, at 5:45 PM, Fauci, Anthony (NIH/NIAID) [E] (b)(6) wrote:

FYI

From: Alison Galvani (b)(6)
Sent: Monday, April 30, 2018 9:47 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Pratha Sah (b)(6) Meagan Fitzpatrick (b)(6)
Subject: Re: Tomorrow AM

Simpler:



On Mon, Apr 30, 2018 at 8:55 PM, Alison Galvani (b)(6) wrote:

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<image001.png>
These figures show the age-stratified impact of a universal vaccine on hospitalizations and deaths. We considered a scenario in which a total of 150 million vaccines are distributed, but the percentage of universal vaccination varies. As the contribution of the universal vaccine increases, hospitalizations and deaths are markedly reduced.

Here we assumed that the efficacy of universal is 75% and season vaccine is 48.5% (median of past six years), respectively. Even with our assumption that the universal vaccine does not protect against influenza B (while the seasonal does), the benefits of the a universal vaccine are substantial.

On Mon, Apr 30, 2018 at 8:01 PM, Alison Galvani (b)(6) wrote:
No worries. Of course I completely understand. As it happens we have been modeling the epidemiological effectiveness of your proposed universal vaccine. If you think that might be helpful, I'd be happy to send some preliminaries results.

Sent from my iPhone

On Apr 30, 2018, at 7:45 PM, Fauci, Anthony (NIH/NIAID) [E] (b)(6) wrote:

Alison:
I am very sorry, but I just found out a few minutes ago (welcome to my life) that I have to prepare a briefing paper on universal influenza vaccines for Secretary Azar to discuss with the President. I am mobilizing my staff and we are going to work on it tonight separately and gather very early in the AM to finalize it so that the Secretary can have it by mid-morning. Therefore, I will regrettably have to cancel our 8:00 AM meeting. I am sorry if this causes you any inconvenience. Let us connect next time around.
Thanks,
Tony
Anthony S. Fauci, MD
Director
National Institute of Allergy and Infectious Diseases
Building 31, Room 7A-03
31 Center Drive, MSC 2520
National Institutes of Health
Bethesda, MD 20892-2520
Phone: (b)(6)
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AMERICAN OVERSIGHT

Alison Galvani, PhD

Director, Yale Center for Infectious Disease Modeling and Analysis (CIDMA)
Burnett and Stender Families Professor of Epidemiology
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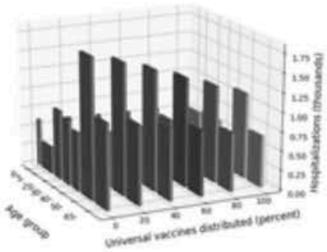

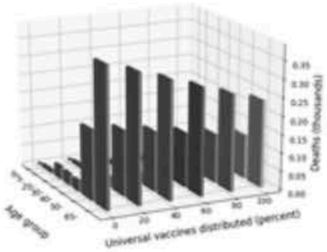

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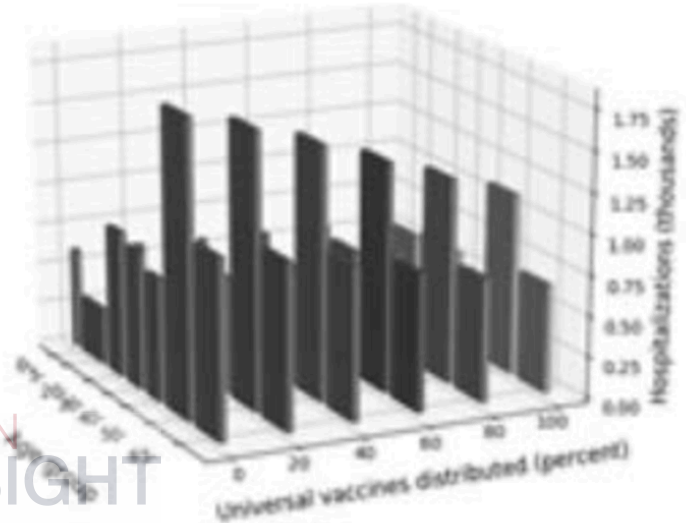

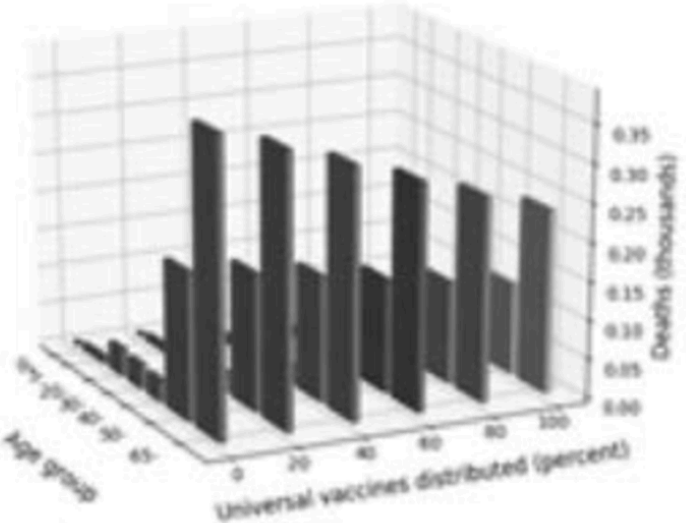

AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Tue, 1 May 2018 22:00:46 +0000
To: Paules, Catharine (NIH/NIAID) [E]
Subject: RE: Biondvax info

Thanks, Cat.

From: Paules, Catharine (NIH/NIAID) [E]
Sent: Tuesday, May 01, 2018 1:06 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Folkers, Greg (NIH/NIAID) [E] (b)(6) Billet, Courtney (NIH/NIAID) [E] (b)(6) Auchincloss, Hugh (NIH/NIAID) [E] (b)(6) Hudgings, Carole (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6) Eisinger, Robert (NIH/NIAID) [E] (b)(6) Marston, Hilary (NIH/NIAID) [E] (b)(6)
Subject: Biondvax info

Hi Dr. Fauci,

(b)(5)

From: Roberts, Chris (NIH/NIAID) [E]
Sent: Thursday, November 02, 2017 1:00 PM
To: Paules, Catharine (NIH/NIAID) [E] (b)(6)
Cc: Erbelding, Emily (NIH/NIAID) [E] (b)(6) Eisinger, Robert (NIH/NIAID) [E] (b)(6) Marston, Hilary (NIH/NIAID) [E] (b)(6) Mulach, Barbara (NIH/NIAID) [E] (b)(6) Abbey, Lillian (NIH/NIAID) [E] (b)(6) Post, Diane (NIH/NIAID) [E] (b)(6) Mason, Robin (NIH/NIAID) [E] (b)(6) Schuster, Claire (NIH/NIAID) [E] (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6) Knudsen, Tena (NIH/NIAID) [E] (b)(6)
Subject: RE: PR - October2017

Hi Cat,

(b)(5)

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Withheld pursuant to exemption

(b)(5)

of the Freedom of Information and Privacy Act

AMERICAN OVERSIGHT

From: Paules, Catharine (NIH/NIAID) [E]
Sent: Thursday, November 02, 2017 9:58 AM
To: Roberts, Chris (NIH/NIAID) [E] (b)(6)
Cc: Erbelding, Emily (NIH/NIAID) [E] (b)(6) Eisinger, Robert (NIH/NIAID) [E] (b)(6) Marston, Hilary (NIH/NIAID) [E] (b)(6) Mulach, Barbara (NIH/NIAID) [E] (b)(6) Abbey, Lillian (NIH/NIAID) [E] (b)(6) Post, Diane (NIH/NIAID) [E] (b)(6) Mason, Robin (NIH/NIAID) [E] (b)(6) Schuster, Claire (NIH/NIAID) [E] (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6)
Subject: RE: PR - October2017

Hi Chris,
Just wanted to follow up on this to see if you had a copy of the phase 2 protocol and if you have details about the funding of this project (and others with Biondvax if applicable). Thanks much, Cat

AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Tue, 1 May 2018 20:47:50 +0000
To: Marston, Hilary (NIH/NIAID) [E]
Subject: Use this

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Tuesday, May 01, 2018 4:44 PM
To: Marston, Hilary (NIH/NIAID) [E] (b)(6)
Subject: RE: EOP

From: Marston, Hilary (NIH/NIAID) [E]
Sent: Tuesday, May 01, 2018 10:06 AM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Conrad, Patricia (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6) Paules, Catharine (NIH/NIAID) [E] (b)(6)
Subject: EOP

NSC is working on an executive order on pandemic flu (both modernizing manufacturing and improving breadth/duration). Lu reached out to get your informal read on the following – edits/additions are welcome, but are **needed by COB**. You will have more formal opportunities to weigh in as it moves through interagency etc, but for obvious reasons, she wanted to consult you sooner. This came to me as the NSC interagency staff level contact. Please see below:

Modernizing America's influenza vaccine manufacturing enterprise to promote public health and national security

Section 2. *Policy*. Modernizing the flu vaccine enterprise is a public health and national security priority. To advance the development and appropriate use of flu vaccines, the United States will:

a. Accelerate research to develop next-generation flu vaccines that are broadly protective against many or all flu strains;

b. Reduce reliance on chicken-egg-based flu vaccine production by scaling-up alternative production methods that are faster and more reliable; and

c. Increase flu vaccine use through enhanced communication and information efforts.

NIH:

AMERICAN OVERSIGHT

a. Has developed a **strategic plan and** research agenda toward the development of flu vaccines that can protect individuals over many years and against multiple types of influenza (a "universal vaccine").
b. Will provide estimated cost and timelines **for their strategic plan and research agenda**
c. **Will coordinate with CDC, BARDA and FDA in the implementation of the research plan.**

Hilary Marston, MD, MPH
Medical Officer and Policy Advisor for Global Health
NIAID Office of the Director
(b)(6)
(b)(6) (cell)

AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Tue, 1 May 2018 20:43:51 +0000
To: Marston, Hilary (NIH/NIAID) [E]
Subject: RE: EOP

From: Marston, Hilary (NIH/NIAID) [E]
Sent: Tuesday, May 01, 2018 10:06 AM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Conrad, Patricia (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6) Paules, Catharine (NIH/NIAID) [E] (b)(6)
Subject: EOP

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NIH will…

a. Develop a **strategic plan and** research agenda, with estimated cost and timelines toward the development of flu vaccines that can protect individuals over many years and against multiple types of influenza (a "universal vaccine").
b. **Coordinate with CDC, BARDA and FDA in the implementation of the research plan.**

Hilary Marston, MD, MPH
Medical Officer and Policy Advisor for Global Health
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AMERICAN OVERSIGHT

(b)(6)

(b)(6) (cell)

AMERICAN OVERSIGHT

From: Marston, Hilary (NIH/NIAID) [E]
Sent: Tue, 1 May 2018 14:05:32 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Conrad, Patricia (NIH/NIAID) [E];Vance, Meaghan (NIH/NIAID) [C];Folkers, Greg (NIH/NIAID) [E];Paules, Catharine (NIH/NIAID) [E]
Subject: EOP

NSC is working on an executive order on pandemic flu (both modernizing manufacturing and improving breadth/duration). Lu reached out to get your informal read on the following – edits/additions are welcome, but are **needed by COB**. You will have more formal opportunities to weigh in as it moves through interagency etc, but for obvious reasons, she wanted to consult you sooner. This came to me as the NSC interagency staff level contact. Please see below:

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NIH will…

a. Develop a research agenda, with estimated cost and timelines, in coordination with BARDA, FDA, and CDC, toward the development of flu vaccines that can protect individuals over many years and against multiple types of influenza (a "universal vaccine")

Hilary Marston, MD, MPH
Medical Officer and Policy Advisor for Global Health
NIAID Office of the Director
(b)(6)
(b)(6) (cell)

AMERICAN OVERSIGHT

From: Jeffrey V. Ravetch
Sent: Tue, 1 May 2018 13:22:31 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Vaccines effect
Attachments: DiLillo, Cell.pdf, HIV vaccinal.pdf

Tony,
Thanks again for meeting with us yesterday. We greatly appreciate all you've done for the community and continue to do for our research efforts. We're very grateful.
Getting back to our discussion of Malcolm's paper on inducing memory CD8 cells by antibody treatment, I've attached the Cell paper we published describing this phenomenon and the mechanism that we believe induces this response. I've also attached a figure copied from a review that Malcolm wrote last year on his study demonstrating this effect in HIV infected macaques. I believe this pathway is a general one and would be relevant in flu as well as HIV. We have developed the reagents and assay systems to optimize the Fc of bNAbs to induce this effect and would be happy to discuss working with you and the VRC to bring these optimized antibodies to the clinic.
Again, thanks for the meeting yesterday. I look forward to continuing our discussion on flu and HIV.
Jeff

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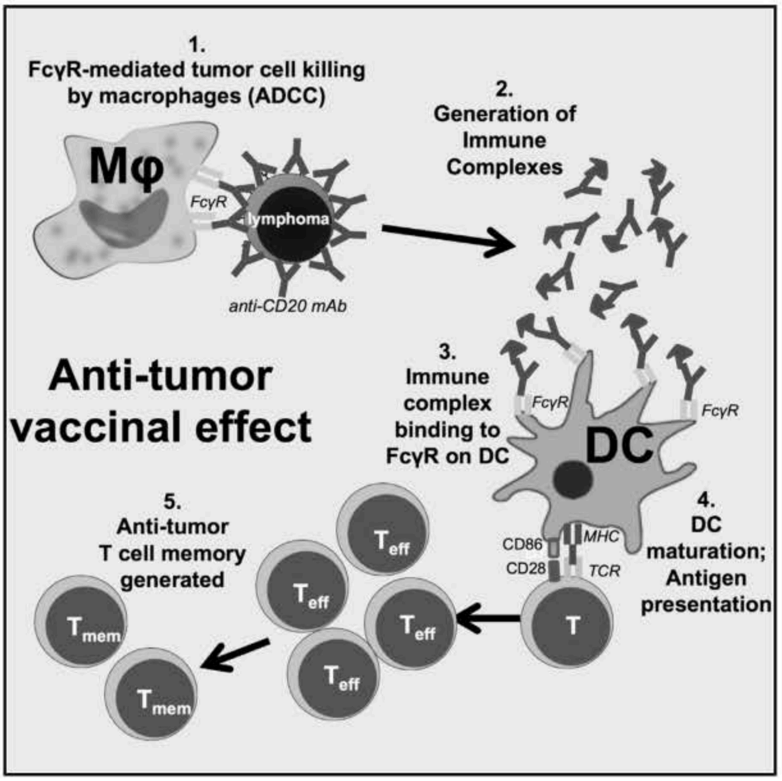
Article

Cell

Differential Fc-Receptor Engagement Drives an Anti-tumor Vaccinal Effect

Graphical Abstract



Authors

David J. DiLillo, Jeffrey V. Ravetch

Correspondence

ravetch@rockefeller.edu

In Brief

Cytotoxic antibodies targeting tumor cells can prime the cellular immune system for the generation of anti-tumor T cell memory. Tumor antigen immune complexes engage FcγRs expressed by CD11c^{+} antigen-presenting cells to stimulate this anti-tumor vaccinal effect.

Highlights

- Tumor-bearing mice given cytotoxic anti-tumor antibody develop T cell tumor immunity
- This anti-tumor vaccinal effect is differentially driven by two distinct human FcγRs
- ADCC-mediated tumor clearance requires engagement of hFcγRIIIA on macrophages
- Selective engagement of hFcγRIIA on DCs promotes long-term immunity against cancer

DiLillo & Ravetch, 2015, Cell *161*, 1–11
May 21, 2015 ©2015 Elsevier Inc.
http://dx.doi.org/10.1016/j.cell.2015.04.016

CellPress

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HHS-NIAID-20-0593-A-001146

Please cite this article in press as: DiLillo and Ravetch, Differential Fc-Receptor Engagement Drives an Anti-tumor Vaccinal Effect, Cell (2015), http://dx.doi.org/10.1016/j.cell.2015.04.016

Differential Fc-Receptor Engagement Drives an Anti-tumor Vaccinal Effect

David J. DiLillo[1] and Jeffrey V. Ravetch[1,*]
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*Correspondence: ravetch@rockefeller.edu
http://dx.doi.org/10.1016/j.cell.2015.04.016

SUMMARY

Passively administered anti-tumor monoclonal antibodies (mAbs) rapidly kill tumor targets via FcγR-mediated cytotoxicity (ADCC), a short-term process. However, anti-tumor mAb treatment can also induce a vaccinal effect, in which mAb-mediated tumor death induces a long-term anti-tumor cellular immune response. To determine how such responses are generated, we utilized a murine model of an anti-tumor vaccinal effect against a model neoantigen. We demonstrate that FcγR expression by CD11c$^+$ antigen-presenting cells is required to generate anti-tumor T cell responses upon ADCC-mediated tumor clearance. Using FcγR-humanized mice, we demonstrate that anti-tumor human (h)IgG1 must engage hFcγRIIIA on macrophages to mediate ADCC, but also engage hFcγRIIA, the sole hFcγR expressed by human dendritic cells (DCs), to generate a potent vaccinal effect. Thus, while next-generation anti-tumor antibodies with enhanced binding to only hFcγRIIIA are now in clinical use, ideal anti-tumor antibodies must be optimized for both cytotoxic effects as well as hFcγRIIA engagement on DCs to stimulate long-term anti-tumor cellular immunity.

INTRODUCTION

Passive administration of anti-tumor antibodies is an important clinical tool for the management of a variety of cancers (Pincetic et al., 2014) and generally functions by targeting malignant cells through Fc-receptor for IgG (FcγR)-mediated antibody-dependent cellular cytotoxicity (ADCC) by myeloid effector cells (Clynes et al., 2000; Taylor and Lindorfer, 2008; Uchida et al., 2004) or possibly natural killer (NK) cells. Because of this FcγR-mediated mechanism of action, next-generation versions of anti-tumor mAbs that have been Fc-engineered for enhanced engagement of activating hFcγRIIIA are now being used in the clinic or are under investigation (Goede et al., 2014). However, while ADCC-mediated tumor killing is rapid and relatively short-acting, patients with some malignancies see long-term responses after cessation of antibody therapy; this has prompted the hypothesis that a vaccinal or auto-immunization effect is initiated, in which tumor targeting by a monoclonal antibody (mAb) primes the patient's immune system to generate an anti-tumor T cell memory response (Cartron et al., 2004). Thus, it has been demonstrated that cellular immune responses are generated in both mice and patients treated with anti-HER-2/neu mAb (Park et al., 2010; Taylor et al., 2007). Anti-MUC1 cellular immune responses have also been reported after the use of anti-MUC1 mAb in patients with MUC1$^+$ tumors (de Bono et al., 2004). Evidence in lymphoma patients suggests that a vaccinal effect can be generated by anti-human (h)CD20 mAb immunotherapy (rituximab), since a single course of treatment with mAb can result in long-lasting, durable responses (Cartron et al., 2004). In support of this, it has been reported that some patients treated with rituximab developed lymphoma-specific anti-idiotype T cell responses after mAb treatment (Hilchey et al., 2009). Recent studies in mice have also demonstrated that passive administration of anti-CD20 mAbs can initiate anti-tumor cellular immune responses (Abès et al., 2010). Therefore, while the hypothesis of a tumor-specific antibody-induced anti-tumor vaccinal effect has persisted for more than a decade, an experimentally derived mechanistic explanation is lacking.

New technologies have enabled the identification of tumor mutational signatures, some common across multiple cancer types while others are restricted to specific malignancies (Alexandrov et al., 2013). Thus, mutation-induced, developmentally restricted, or overexpressed tumor neoantigens are a major target of tumor-infiltrating lymphocytes in patients (Fritsch et al., 2014; Tran et al., 2014). Neoantigen-specific CD4$^+$ and CD8$^+$ T cells have been identified, showing that such antigens are indeed processed and presented (Gros et al., 2014; van Rooij et al., 2013). Further, new immune-checkpoint blockade therapies function in patients by amplifying neoantigen-specific responses (van Rooij et al., 2013). However, although studies analyzing antibody responses to tumor neoantigens are lacking, antibody:antigen immune complexes can stimulate cellular immunity by engaging activating FcγRs on antigen-presenting cells, such as dendritic cells (DCs), to induce DC maturation, traditional antigen presentation and cross-presentation, co-stimulatory molecule upregulation, and stimulate cellular immune responses in both mice (Kalergis and Ravetch, 2002; Rafiq et al., 2002) and humans (Boruchov et al., 2005; Dhodapkar et al., 2005). Often, antibody:antigen immune complex immunization results in more potent cross-presentation and CD4 or CD8 T cell responses than antigen immunization alone. Thus, a logical approach to boosting cellular immune responses involves passive administration of antibodies reactive with tumor antigens or tumor neoantigens. Therefore, in this current study, we utilize a tumor model expressing a model tumor neoantigen to

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HHS-NIAID-20-0593-A-001147

test whether and how passive anti-tumor antibody treatment stimulates an anti-tumor vaccinal effect and cellular immune response.

Three activating FcγRs are expressed in mice (mouse [m]FcγRI, mFcγRIII, and mFcγRIV) and humans (hFcγRI, hFcγRIIA, and hFcγRIIIA), and a single inhibitory FcγR, FcγRIIB, is expressed in both species. The cellular outcome of IgG interactions with FcγRs is governed by the affinity of an antibody's Fc for the specific receptor and the expression pattern of those receptors on effector cells (Nimmerjahn and Ravetch, 2008). Since most effector cells co-express activation and inhibitory FcγRs, it is the ratio of the binding affinities of a specific IgG Fc to these receptors that will determine the outcome of the IgG-FcγR interaction. These binding affinities are determined by the amino acid sequences of the IgG Fc subclasses and the IgG Fc's N-linked glycan. The IgG Fc composition can dramatically influence the in vivo outcome of engaging a tumor antigen by directing the antibody-antigen immune complex or opsonized cell into either a pro- or anti-inflammatory response. For example, mIgG2a antibodies trigger cytotoxicity by virtue of this Fc having a 2-log greater affinity for the activating mFcγRIV receptor as compared to the inhibitory mFcγRIIb receptor, while mIgG1 preferentially engages the inhibitory mFcγRIIb receptor.

Mice and humans differ regarding the specific FcγRs expressed on various antigen-presenting cells and the relative affinities of each IgG Fc subclass for each FcγR (Nimmerjahn and Ravetch, 2007). Thus, hIgG1 Fc does not preferentially engage a single hFcγR, as occurs in mice. Two low-affinity activating FcγRs are expressed on monocytes and macrophages in both mice (mFcγRIII and mFcγRIV) and humans (hFcγRIIA and hFcγRIIIA). Murine NK cells express only mFcγRIII, while human NK cells express only hFcγRIIIA. Importantly, while mice express two low-affinity activating FcγRs, mFcγRIII and mFcγRIV, on DCs, humans only express one low-affinity activating FcγR on DCs, hFcγRIIA. Because of these species differences, we have generated FcγR-humanized mice, which express the full array of hFcγRs on a background lacking all murine FcγRs (Smith et al., 2012). Appropriate cell-type-specific expression of all hFcγRs is observed in the FcγR-humanized mice, allowing the characterization of hIgG1 antibody-mediated effector function in the context of human FcγRs.

Here, using a murine lymphoma expressing a model tumor neoantigen (hCD20), we mechanistically dissect how an anti-tumor cellular immune response is generated after passive treatment of lymphoma-bearing mice with anti-hCD20 mAb. We demonstrate that not only are activating FcγRs required for macrophage-mediated ADCC, but activating FcγR expression specifically on CD11c$^+$ antigen-presenting cells is required for the generation of an anti-tumor T cell memory immune response and long-term survival. Because of the complexity of the FcγR system, with multiple genes expressed and regulated differentially on the diverse cells of the immune system, designing an anti-tumor antibody for optimal activity requires compatible model systems. We therefore assessed the generation of an anti-tumor vaccinal effect for a hIgG1 anti-hCD20 mAb in FcγR-humanized mice. We show that anti-tumor mAb must engage hFcγRIIIA on clodronate liposome (CLOD)-sensitive macrophages to mediate immediate ADCC, as well as hFcγRIIA (the sole FcγR expressed by human DCs) in order to stimulate a long-term anti-tumor cellular immune response. Thus, while next-generation anti-tumor antibodies with enhanced binding to only hFcγRIIIA on innate effector cells are now in clinical use, our results indicate that anti-tumor antibodies with optimal long-term survival benefit must be optimized for both their short-acting cytotoxic effects through hFcγRIIIA, as well as for engagement of hFcγRIIA on DCs to stimulate long-term anti-tumor cellular immunity.

RESULTS

Generation of an Anti-tumor Vaccinal Effect by Passive Anti-tumor Antibody Treatment

To understand how passive anti-tumor mAb treatment can induce long-term anti-tumor cellular immune responses, we adapted a murine lymphoma model (Abès et al., 2010) of an anti-neoantigen mAb-mediated vaccinal effect (Figure 1A). Wild-type C57BL/6 mice were given syngenic EL4 lymphoma cells that express hCD20 as a tumor neoantigen (EL4-hCD20 cells), followed by mIgG2a isotype anti-hCD20 mAb. The mIgG2a isotype preferentially engages activating mFcγRs and is the most potent mouse subclass for triggering effector cells to result in cellular cytotoxicity, phagocytosis, and inflammatory responses (Nimmerjahn and Ravetch, 2005; Pincetic et al., 2014). Mice receiving lymphoma cells plus anti-hCD20 mAb clear the tumors and survive in an activating FcγR-dependent manner; wild-type mice survive the tumor challenge, while FcRα null (Smith et al., 2012) mice (that lack all mFcγRs: mFcγRI, mFcγRIIB, mFcγRIII, and mFcγRIV) and *Fcer1g*$^{-/-}$ mice (that lack all activating mFcγRs: mFcγRI, mFcγRIII, and mFcγRIV) do not (Figures 1B and S1A). The activating mFcγRIV is a major contributor during this clearance of tumor cells, since only 52% of *Fcgr4*$^{-/-}$ mice survive tumor challenge after anti-hCD20 mAb treatment ($p < 0.001$; Figure S1B). Non-FcγR binding DA265 mutant anti-hCD20 was also unable to clear tumors (Figure S1C). Thus, activating FcγRs mediate the initial ADCC clearance of tumor cells by anti-tumor mAb, as reported (Clynes et al., 2000; Uchida et al., 2004).

We next assessed whether an anti-tumor vaccinal effect was generated in mice that survived the initial EL4-hCD20 challenge via treatment with anti-hCD20 mAb. Ninety days after the initial challenge when anti-hCD20 mAb had been cleared (mIgG2a half-life = 7 days) (Vieira and Rajewsky, 1988), surviving tumor/mAb-primed mice were re-challenged with 5×10^6 EL4-hCD20 tumor cells, a dose that is 10-fold greater than the initial challenge, without treatment with any additional anti-hCD20 mAb. These primed mice showed 100% survival during re-challenge with EL4-hCD20 cells (Figure 1C). By contrast, surviving tumor/mAb-primed mice re-challenged with EL4-WT cells, which do not express hCD20, showed poor survival. Similar results were seen using a different anti-hCD20 mAb, clone 2B8, which is the parental hybridoma from which rituximab was generated (Figures S1D and S1E). Thus, mice primed with EL4-hCD20 and anti-hCD20 mAb generate a memory immune response and subsequently reject re-challenge with EL4-hCD20 cells, but not EL4 WT cells.

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HHS-NIAID-20-0593-A-001148

Please cite this article in press as: DiLillo and Ravetch, Differential Fc-Receptor Engagement Drives an Anti-tumor Vaccinal Effect, Cell (2015), http://dx.doi.org/10.1016/j.cell.2015.04.016

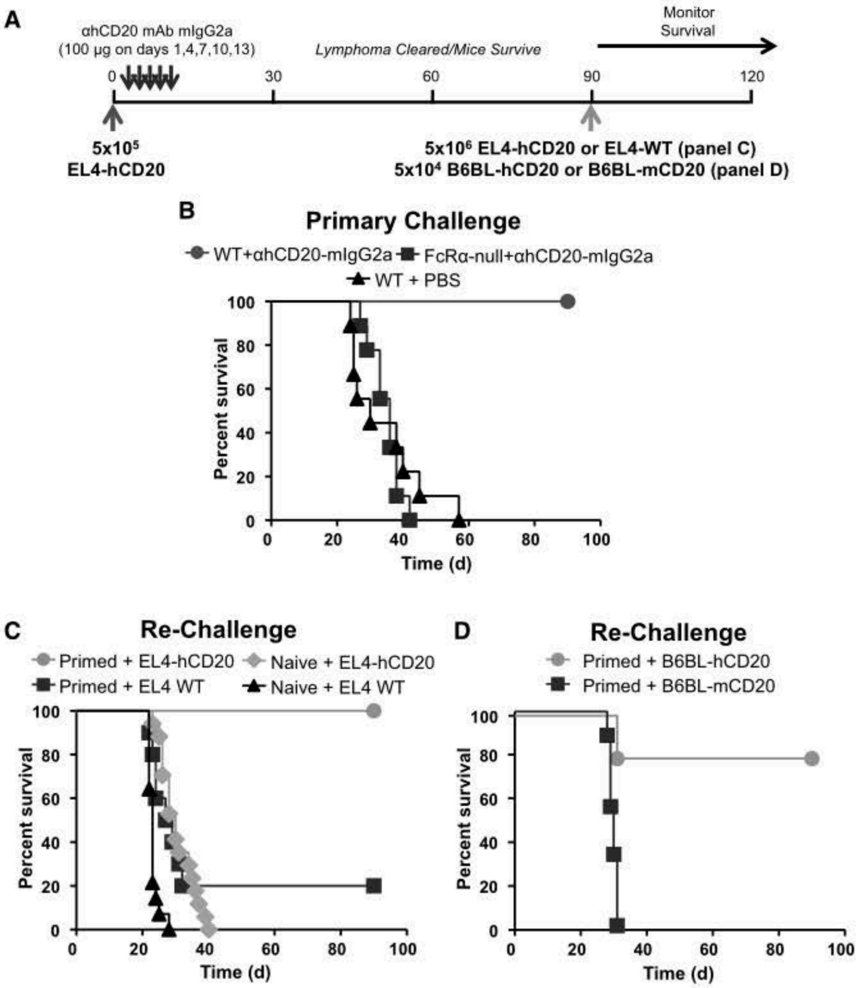

Figure 1. Fc-FcγR Interactions Are Required for the Clearance of Lymphoma by mAb and the Initiation of an Anti-tumor Vaccinal Effect

(A) Experimental protocol. Mice were injected i.v. with 5×10^5 EL4-hCD20 lymphoma cells on day 0 (red arrow) and received 100 μg of mIgG2a isotype anti-hCD20 mAb (clone CAT13.6E12) on days 1, 4, 7, 10, and 13 (blue arrows). On day 90, surviving mice were re-challenged i.v. with 5×10^6 EL4-hCD20 lymphoma cells (green arrow) or EL4-WT cells, a 10-fold greater dose of tumor compared to the primary lymphoma challenge, and survival was monitored daily. Alternatively, surviving mice were re-challenged with 5×10^4 B6BL-hCD20 or B6BL-mCD20 cells i.v.

(B) Wild-type (red circles) or FcRα null mice (blue squares) were injected with EL4-hCD20 cells and treated with mIgG2a isotype anti-hCD20 mAb, with survival assessed daily (n = 9–11 mice per group).

(C) After 90 days, surviving (primed) mice treated with mIgG2a anti-hCD20 mAb from (B) were re-challenged with EL4-hCD20 cells (green circles) or EL4-WT cells (blue squares) with survival assessed daily. For comparison, naive mice were also challenged with EL4-hCD20 cells (gray diamonds) or EL4-WT cells (filled triangles). n = 10–13 mice per group.

(D) Mice were primed with EL4-hCD20 lymphoma cells and mAb as in (A) and (B) before re-challenge on day 90 with B6BL tumor cells expressing either hCD20 (green circles) or mCD20 (blue squares). n = 10 mice per group.

In this model of the vaccinal effect, both $CD4^+$ and $CD8^+$ T cells are required after antibody treatment in order to reject the tumor re-challenge (Abès et al., 2010). Specifically, T cell depletion studies demonstrated that $CD4^+$ T cells are required during the initial phases of antibody therapy as well as during tumor re-challenge in order to reject tumors. Using CD8-deficient mice, it was also shown that $CD8^+$ cells are required for tumor rejection during re-challenge. By contrast, mice do not mount any detectable antibody response against hCD20 or EL4-hCD20 cells, and adoptive transfer of serum from tumor/mAb-primed mice does not protect against tumor challenge (Abès et al., 2010). In Figure 1C, we show that EL4-hCD20/anti-hCD20 mAb-primed mice only reject EL4-hCD20 cells, and not wild-type EL4 cells, suggesting that the vaccinal effect is directed against hCD20, with little detectable antigen spreading. To confirm that at least a portion of the vaccinal effect cellular immune response is directed at hCD20, mice primed with EL4-hCD20 cells and anti-hCD20 mAb were re-challenged with a distinct tumor cell line, B6BL lymphoma cells, that expressed either cell-surface hCD20 or an irrelevant antigen (mCD20). While 100% of mice re-challenged with B6BL-mCD20 died by day 31, 80% of mice re-challenged with B6BL-hCD20 cells survived at least 90 days (Figure 1D; $p = 0.0001$). Thus, only cells expressing hCD20 were capable of being rejected. Collectively, these experiments demonstrate that an anti-hCD20 immune response is generated after the initial FcγR-mediated clearance of tumor cells by ADCC.

Expression of mFcγRIV on $CD11c^+$ Cells Is Required for the Generation of an Anti-tumor Vaccinal Effect

To understand the mechanistic basis for an anti-tumor vaccinal effect and to determine whether FcγR expression plays a role during this process, we utilized mice with a $CD11c^+$ cell-specific deletion of mFcγRIV. *Fcgr4*$^{fl/fl;cd11c\text{-}cre}$ mice (Nimmerjahn et al., 2010) show a complete absence of mFcγRIV expression on spleen $CD11c^{hi}$ DCs, but only a partial decrease in mFcγRIV expression on spleen $CD11b^+Gr\text{-}1^{lo}SSC^{lo}$ monocytes (Figure 2A). All $CD11c^{hi}$ cells lose mFcγRIV expression in *Fcgr4*$^{fl/fl;cd11c\text{-}cre}$ mice, while spleen $CD11c^{int}$ cells show partial loss of mFcγRIV and $CD11c^-$ cells show only a modest decrease in mFcγRIV (Figure S2A; Table S1). The majority of $CD11c^{hi}CD8^+$ DCs and a fraction of $CD11c^{hi}CD4^+$ DCs express mFcγRIV, both of which lose all mFcγRIV expression in *Fcgr4*$^{fl/fl;cd11c\text{-}cre}$ mice (Figure S2E). While the majority of $CD11c^{int/-}CD11b^{int}$ cells lose expression of mFcγRIV, only a modest reduction in mFcγRIV is seen in $CD11c^{int/-}CD11b^{hi}$ cells in *Fcgr4*$^{fl/fl;cd11c\text{-}cre}$ mice (Figure S2B). Expression of mFcγRIV is not affected in

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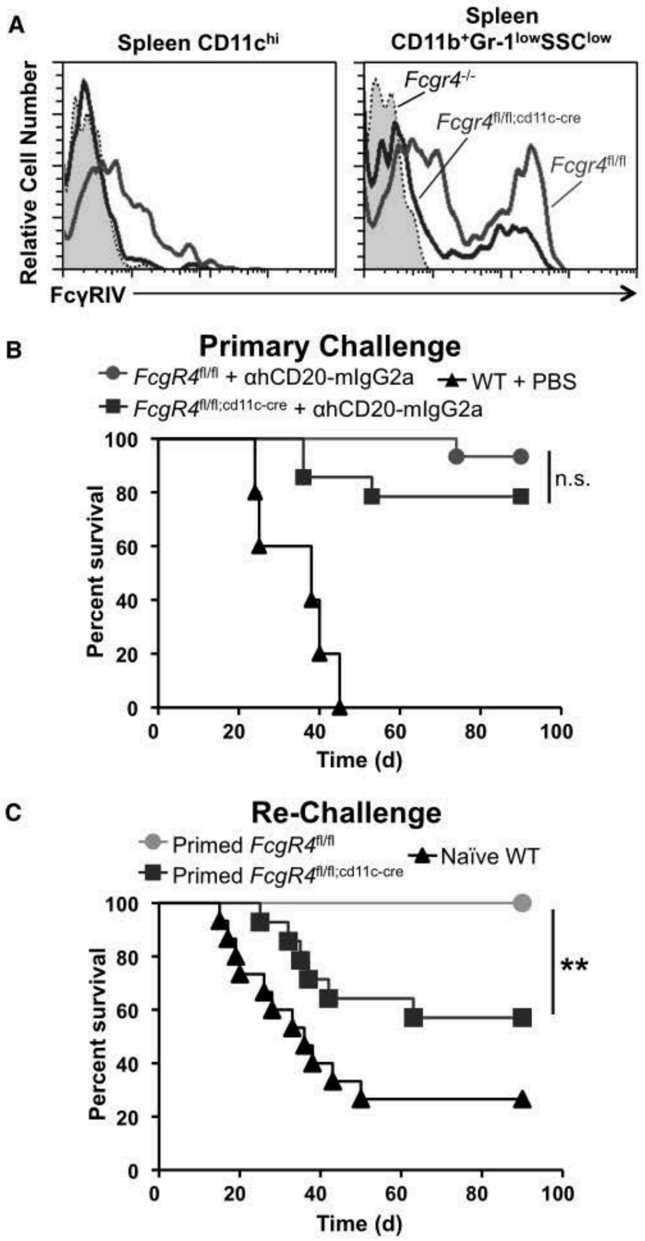

Figure 2. Expression of mFcγRIV on CD11c$^+$ Cells Is Required for the Generation of an Anti-tumor Vaccinal Effect

(A) mFcγRIV expression levels on spleen innate cell subsets. Spleen lymphocytes were harvested from *Fcgr4*$^{fl/fl}$ (red line), *Fcgr4*$^{fl/fl;cd11c\text{-}cre}$ (blue line), or *Fcgr4*$^{-/-}$ (shaded line) mice, and mFcγRIV expression levels on CD11c$^+$ dendritic cells and CD11b$^+$Gr-1^{low}SSC^{low} resident monocytes was assessed. Representative flow cytometry histograms from three independent experiments are shown.

(B) *Fcgr4*$^{fl/fl}$ (red circles; n = 15) or *Fcgr4*$^{fl/fl;cd11c\text{-}cre}$ (blue squares; n = 14) mice were given EL4-hCD20 cells and treated with mIgG2a anti-hCD20 mAb, with survival monitored daily.

(C) After 90 days, surviving *Fcgr4*$^{fl/fl}$ (green circles) or *Fcgr4*$^{fl/fl;cd11c\text{-}cre}$ (blue squares) mice treated with mIgG2a isotype anti-hCD20 mAb from (B) were re-challenged with EL4-hCD20 cells, with survival assessed daily (n = 14–16 mice per group). Significant differences between groups are indicated: **p = 0.0065; n.s., not significant.

See also Table S1.

Ly6G$^+$ neutrophils (Figure S2C). Most CD11b$^+$CD11c$^{int/-}$F4/80^{hi} macrophages lose mFcγRIV expression in *Fcgr4*$^{fl/fl;cd11c\text{-}cre}$ mice, as these cells express CD11c at intermediate levels (Figure S2D). Similar results demonstrating decreases in mFcγRIV expression on CD11c$^+$ cells and lesser decreases in CD11b$^+$ or F4/80$^+$ cells were seen in bone marrow, peritoneal cavity, and peripheral blood from *Fcgr4*$^{fl/fl;cd11c\text{-}cre}$ mice (Figures S3, S4, and S5; Table S1). Therefore, because mFcγRIV expression was maintained to a sufficient degree on ADCC-mediating innate cells, both control *Fcgr4*$^{fl/fl}$ and *Fcgr4*$^{fl/fl;cd11c\text{-}cre}$ mice were able to clear primary EL4-hCD20 lymphoma cell challenge after treatment with anti-hCD20 mAb (Figure 2B), indicating that mFcγRIV expression on CD11c$^+$ cells is not required for anti-hCD20 mAb-mediated ADCC.

To assess the generation of the anti-hCD20 vaccinal effect in the context of CD11c$^+$ cells lacking mFcγRIV, surviving tumor/mAb-primed *Fcgr4*$^{fl/fl}$ and *Fcgr4*$^{fl/fl;cd11c\text{-}cre}$ mice were re-challenged with EL4-hCD20 cells. Re-challenge of primed *Fcgr4*$^{fl/fl}$ mice resulted in 100% survival (Figure 2C). By contrast, only 57% of tumor/mAb-primed *Fcgr4*$^{fl/fl;cd11c\text{-}cre}$ mice survived re-challenge with EL4-hCD20 cells ($p = 0.0069$). It is likely that activating mFcγRIII, which is also expressed on murine DCs, compensates in the absence of mFcγRIV, thereby explaining why a modest vaccinal effect remains in *Fcgr4*$^{fl/fl;cd11c\text{-}cre}$ mice. Thus, expression of the IgG2a-preferential activating mFcγRIV on CD11c$^+$ antigen-presenting cells is required for the generation of the anti-tumor vaccinal effect after mAb-mediated killing of tumor cells.

CD11c$^+$ Cell Expression of mFcγRIV Is Required to Generate Anti-tumor Cellular Immunity

To quantify anti-tumor cellular immunity in vivo, we performed adoptive transfer experiments (Figure 3A). Mice were given EL4-hCD20 cells and mIgG2a anti-hCD20 mAb, with spleens harvested and total splenocytes or CD3$^+$ T cells isolated and adoptively transferred into naive mice that were then challenged with EL4-hCD20 cells. Adoptive transfer of splenocytes or T cells from naive mice was unable to protect against tumor growth, but transfer of splenocytes or T cells from tumor/mAb-primed mice resulted in 80% ($p = 0.0044$) and 75% ($p = 0.0067$) survival, respectively (Figure 3B). Thus, tumor/mAb-primed mice generate a quantifiable anti-tumor T cell response.

To determine whether DC expression of mFcγRIV is required for the generation of the anti-tumor cellular immune response after passive administration of anti-tumor mAb, tumor/mAb-primed splenocytes from *Fcgr4*$^{fl/fl}$ and *Fcgr4*$^{fl/fl;cd11c\text{-}cre}$ mice were adoptively transferred into naive mice before challenge with EL4-hCD20 cells. While 76% of mice receiving splenocytes from tumor/mAb-primed *Fcgr4*$^{fl/fl}$ mice survived EL4-hCD20 challenge, only 34% of tumor/mAb-primed *Fcgr4*$^{fl/fl;cd11c\text{-}cre}$ mice survived the challenge ($p = 0.0041$; Figure 3C). Thus, expression of mFcγRIV on CD11c$^+$ antigen-presenting cells is required to mediate the generation of an anti-tumor cellular immune response after mAb-mediated clearance of tumor.

hFcγRIIIA Mediates ADCC of hIgG1 mAb-Targeted Tumor Cells in FcγR-Humanized Mice

Fc receptors for mouse IgG are heterogeneous, differing in their binding affinities for IgG subclasses, their expression patterns on

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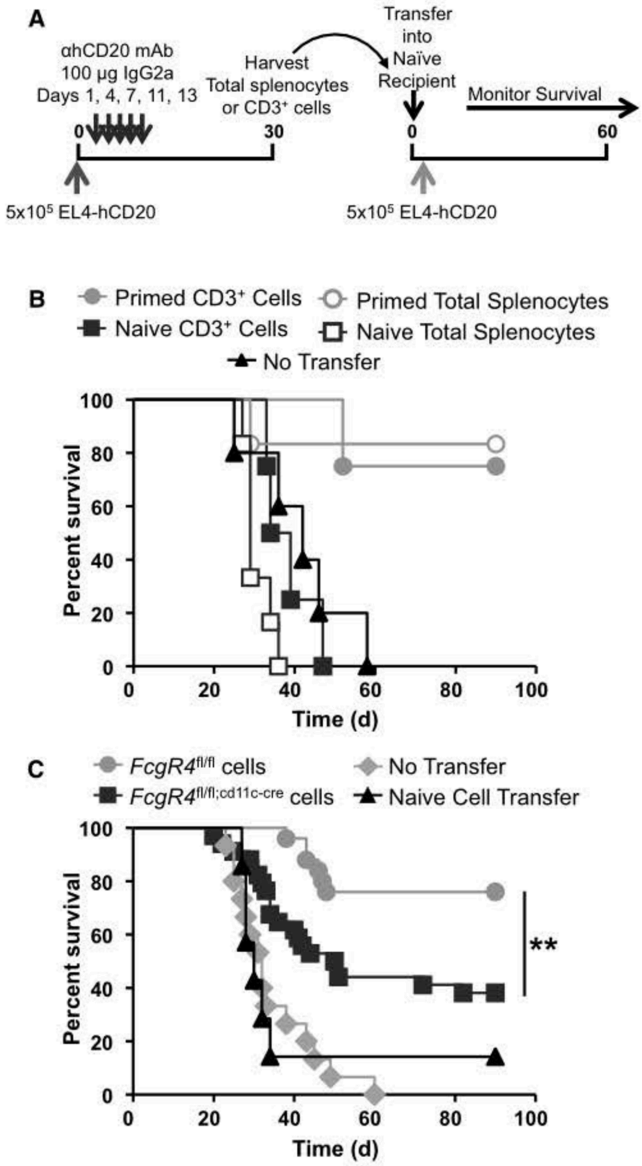

Figure 3. CD11c$^+$ Cell-Specific Expression of mFcγRIV Is Required for the Generation of Anti-tumor Cellular Immunity

(A) Experimental protocol. Mice were injected i.v. with EL4-hCD20 lymphoma cells on day 0 (red arrow) and received mIgG2a anti-hCD20 mAb (blue arrows). On day 30, spleens were harvested and total splenocytes were isolated or CD3$^+$ cells were purified. Then, 50 × 10^6 total splenocytes or 15 × 10^6 CD3$^+$ cells were adoptively transferred into naive mice one day before i.v. challenge with EL4-hCD20 lymphoma cells (green arrow).

(B) Survival was measured in naive mice receiving CD3$^+$ cells (green filled circles) or total splenocytes (green open circles) from tumor and mAb-primed wild-type mice, or CD3$^+$ cells (blue filled squares) or total splenocytes (blue open squares) from naive mice. Another group of naive mice received no adoptive transfer (black triangles). n = 4–6 mice per group.

(C) Survival in naive mice receiving total splenocytes from tumor and mAb-primed *Fcgr4*$^{fl/fl}$ (green circles; n = 25) or *Fcgr4*$^{fl/fl;cd11c\text{-}cre}$ (blue squares, n = 34) mice before EL4-hCD20 cell challenge. Other groups of naive mice received splenocytes from naive mice (black triangles; n = 7) or no adoptive transfer (gray diamonds; n = 15). Significant differences between groups are indicated: **p = 0.0041.

immune cells, and signaling properties (Nimmerjahn and Ravetch, 2006; Pincetic et al., 2014). For example, NK cells in the mouse express only mFcγRIII, a low-affinity activating FcγR. Macrophages and DCs express distinct combinations of both activating (mFcγRI, mFcγRIII, and mFcγRIV) and inhibitory (mFcγRIIB) receptors. Individual mouse subclasses show preferential mFcγR binding affinities, with mIgG1 preferentially engaging the inhibitory mFcγRIIB while mIgG2a engages the activating receptor mFcγRIV with a 2 log higher affinity (Nimmerjahn and Ravetch, 2008, 2011). Thus, selecting an antibody for optimal FcγR engagement requires consideration of the receptors and cell types that are to be engaged.

Further complicating this situation are the inter-species differences between mice and humans. Human FcγR genes, expression patterns, and affinities for the various antibody isotypes differ significantly from mice. Importantly, humans express only the low-affinity activating FcγR—FcγRIIIA—on NK cells, the cells that are thought to primarily mediate cellular cytotoxicity in humans (Seidel et al., 2013), while antigen-presenting DCs express a single, distinct low-affinity activating FcγR, hFcγRIIA (Boruchov et al., 2005; Nimmerjahn and Ravetch, 2008). Therefore, to engineer an antibody to optimize the generation of an anti-tumor vaccinal effect initiated by a hIgG1 antibody in the context of hFcγRs, we utilized FcγR-humanized mice, which express the full array of hFcγRs on a fully immunocompetent C57BL/6 background lacking all mFcγRs (Bournazos et al., 2014a; Smith et al., 2012). FcγR-humanized mice recapitulate hFcγR expression patterns in mouse tissues; spleen CD11c^{hi} DCs express only hFcγRIIA and hFcγRIIB, but do not express hFcγRIIIA, while spleen CD11b$^+$Ly6G$^-$Ly6C^{hi} and CD11b$^+$Ly6G$^-$Ly6C$^{int/-}$ monocytes express hFcγRIIIA, hFcγRIIA and hFcγRIIB in various combinations (Figures 4, S6A, and S6B).

To address the relative contributions of individual hFcγRs during mAb-mediated clearance of primary tumor challenge and during the generation of an anti-tumor vaccinal effect, we generated anti-hCD20 mAb with a hIgG1 Fc backbone and introduced known point mutations (Bournazos et al., 2014b; DiLillo et al., 2014; Smith et al., 2012) that selectively enhance interactions with individual hFcγRs (Figure 5A; Table S2). Thus, the G236A (GA) mutant shows selectively enhanced binding to hFcγRIIA, the A330L/I332E (ALIE) mutant shows selectively enhanced binding to hFcγRIIIA, and the G236A/S239D/A330L/I332E (GASDALIE) mutant shows dramatically enhanced engagement to both hFcγRIIA and hFcγRIIIA.

To determine which hFcγRs are responsible for the initial ADCC-mediated clearance of tumor cells, FcγR-humanized mice were given EL4-hCD20 lymphoma cells and were treated with the various Fc-engineered hIgG1 anti-hCD20 mAbs. The GA mutant anti-hCD20 mAb that selectively engages hFcγRIIA was unable to clear tumor, as 81% of mice receiving this antibody died after primary tumor challenge (Figure 5B). By contrast, 82% and 85% of mice receiving ALIE mutant (selectively engaging hFcγRIIIA) and GASDALIE mutant (selectively enhance both hFcγRIIA and hFcγRIIIA) anti-hCD20 mAb survived the primary EL4-hCD20 tumor challenge, respectively. Further, mice expressing only hFcγRIIA (deficient for murine FcγRs) were unable to clear the primary tumor challenge after mAb

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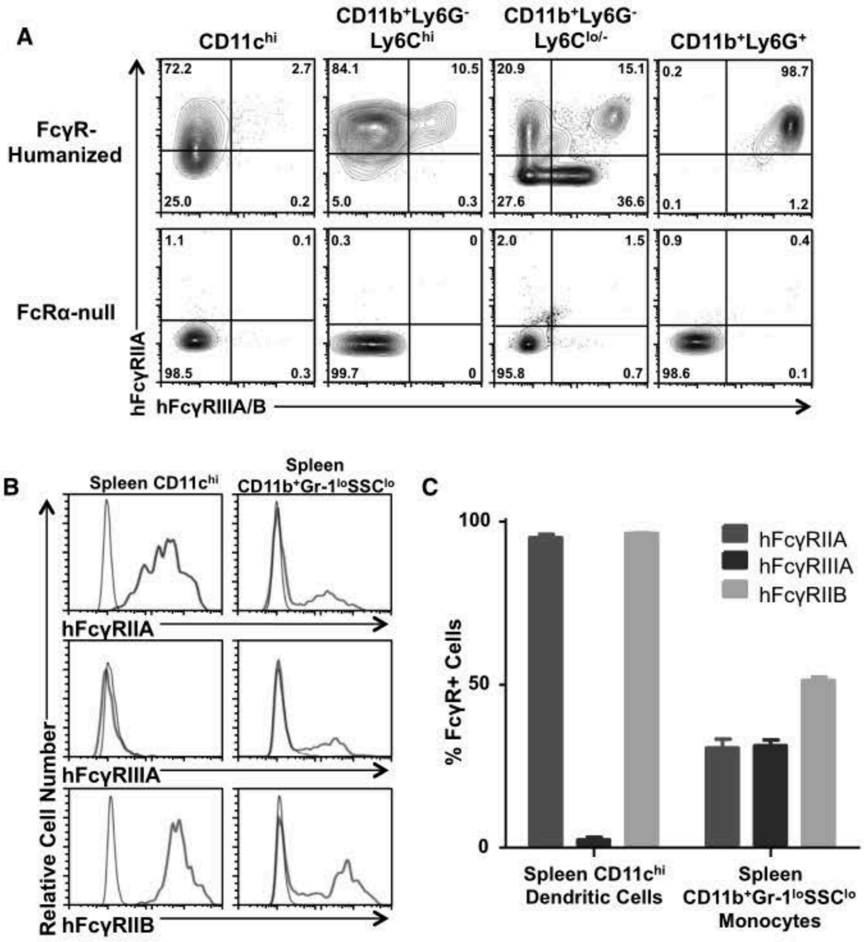

Figure 4. Human FcγR Expression on Innate Cells in FcγR-Humanized Mice

(A) Representative flow cytometry dot plots show hFcγRIIA versus hFcγRIIIA/B expression on spleen cells from FcγR-humanized or FcRα null mice. Numbers represent the frequency of cells in the indicated gate.

(B) DCs and monocytes from FcγR-humanized mouse spleens (red lines) were stained for hFcγRIIA, hFcγRIIIA/B, or hFcγRIIB. Background staining by hFcγR$^-$ cells is shown (gray lines).

(C) Frequencies (±SEM) of hFcγR$^+$ cells among spleen DCs and monocytes (n = 3 per group), with frequencies generated by background staining subtracted.

treatment, while mice expressing only hFcγRIIIA and hFcγRIIIB (deficient for murine FcγRs) showed full survival when treated with GASDALIE mutant anti-hCD20 mAb (Figure 5C). Notably, wild-type hIgG1 anti-hCD20 mAb does not protect FcγR-humanized mice or mice expressing either hFcγRIIA or hFcγRIIIA/B from EL4-hCD20 tumor challenge (Figures S6C and S6D), indicating that wild-type interactions between these hFcγRs and hIgG1 provide insufficient signaling to mediate effector functions at the doses used in this mouse model. Taken together, these results demonstrate that while hFcγRIIA is dispensable, hFcγRIIIA is both necessary and sufficient for mAb-mediated clearance of primary tumor challenge.

Clodronate Liposome-Sensitive Macrophages Mediate ADCC in the Context of hIgG1 and the Human FcγR System

In the context of the mouse FcγR system, clodronate liposome (CLOD)-sensitive macrophages mediate ADCC of antibody-coated target cells (Uchida et al., 2004). NK cells are dispensable for ADCC in this context, presumably because they do not express mFcγRI or mFcγRIV, but only express the low-affinity FcγR, FcγRIII, which interacts with msIgG2a antibodies with ~40-fold lower affinity than mFcγRIV (Otten et al., 2008). However, we now show that hFcγRIIIA mediates ADCC in vivo by hIgG1 antibody. Because hFcγRIIIA is expressed on both NK cells and monocytes/macrophages in FcγR-humanized mice (and humans), we determined whether CLOD-sensitive macrophages mediate ADCC of mAb-coated target cells in the context of the human FcγR system and human IgG1. CLOD decreased total numbers of splenic CD11b^{int}F4/80^{hi} red pulp macrophages by >90% (Figure S6E; $p < 0.0001$). With the exception of CD11c^{hi} DCs (33% depletion, $p = 0.01$), no other cellular populations analyzed were affected by CLOD treatment (Figure S6F).

We first confirmed that depletion of blood and spleen B220$^+$ B cells in hCD20-Tg mice (these mice express hCD20 on mature B cells and also express the full array of murine FcγRs) by mIgG2a isotype anti-hCD20 was dependent on CLOD-sensitive macrophages. Blood and spleen B cell numbers were decreased by 97.5%–98% and 63%–78% ($p < 0.0001$; Figure S7A), respectively, in mice receiving either PBS or control liposomes plus mIgG2a anti-hCD20 mAb. By contrast, no B cells were depleted in mice receiving CLOD plus mIgG2a anti-hCD20 mAb. Thus, as described (Uchida et al., 2004), ADCC of antibody-coated cells in vivo requires CLOD-sensitive macrophage populations.

We next tested the ability of hIgG1 (GASDALIE mutant) anti-hCD20 mAb to deplete hCD20$^+$ B cells in the context of the human FcγR system in hCD20-Tg/FcγR-humanized mice (these mice express hCD20 on mature B cells and also express the full array of human FcγRs, but lack all murine FcγRs), as described above. Blood and spleen B cell numbers were decreased by 86%–90% and 81%–82% ($p < 0.001$), respectively, in hCD20-Tg/FcγR-humanized mice receiving either PBS or control liposomes plus hIgG1 (GASDALIE) anti-hCD20 mAb. By contrast, no B cells were depleted in mice receiving CLOD plus GASDALIE anti-hCD20 mAb (Figure S7B). Similar results were seen in FcγR-humanized mice treated with CLOD and a depleting hIgG1 (GASDALIE) anti-mCD4 mAb (Figure S7C). Therefore, CLOD-sensitive macrophages are required for ADCC mediated by hIgG1 antibody in the context of the human FcγR system.

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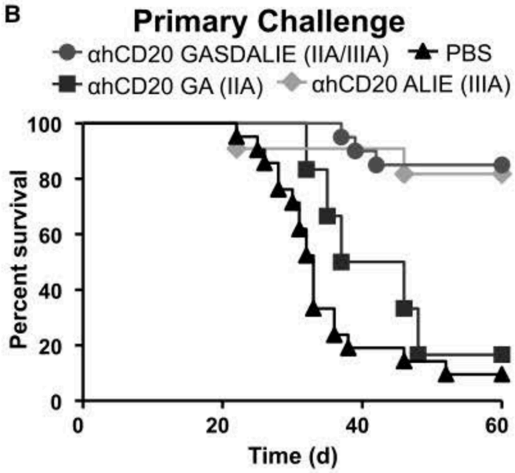
A

Mutant	FcγRIIA	FcγRIIB	FcγRIIIA
huIgG1 (wild-type)	+	+	+
GA	++	+	+
ALIE	+	+	++
GASDALIE	+++	+	+++

B



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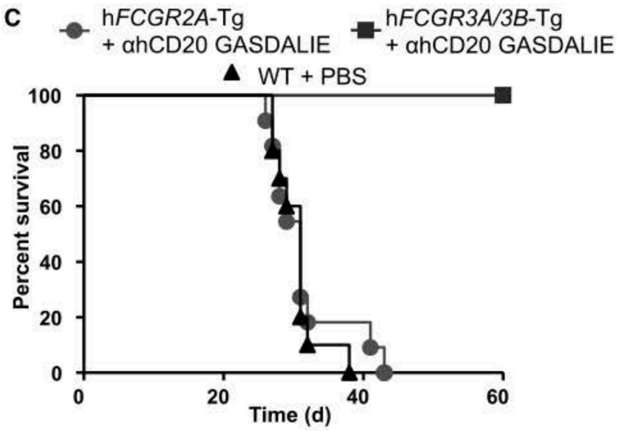

Figure 5. Differential hFcγR Engagement Mediates Tumor Cytotoxicity

(A) Anti-hCD20 hIgG1 mAb Fc mutants for selectively enhanced engagement of hFcγRs. Relative binding capabilities to the indicated hFcγRs are shown, based on binding affinities from biacore experiments (Table S2).

(B) hFcγRIIIA engagement mediates cytotoxic clearance of tumor cells by mAb. FcγR-humanized mice were given EL4-hCD20 cells and treated with hIgG1 mutant versions of anti-hCD20 mAb: GASDALIE mutant (enhanced engagement of hFcγRIIA and hFcγRIIIA; red circles; n = 20), GA mutant (preferential hFcγRIIA engagement; blue squares; n = 6), ALIE mutant (preferential hFcγRIIIA engagement; gray diamonds; n = 12), or PBS (black triangles; n = 17), with survival monitored daily.

(C) hFcγRIIIA is necessary and sufficient to mediate the immediate cytotoxic clearance of EL4-hCD20 lymphoma cells. EL4-hCD20 cells were injected into hFCGR2A-Tg mice that were given GASDALIE mutant anti-hCD20 mAb (filled red circles; n = 11), hFCGR3A/B-Tg mice given GASDALIE mutant anti-hCD20 mAb (filled blue squares; n = 11), or wild-type mice given PBS (filled triangles; n = 10) with survival monitored daily.

hFcγRIIA Engagement by Anti-tumor mAb Mediates the Generation of the Anti-tumor Vaccinal Effect in FcγR-Humanized Mice

Finally, we assessed to what extent the anti-tumor vaccinal effect was generated in FcγR-humanized mice (Figure 6A) treated with anti-hCD20 mAb mutants that selectively engage only hFcγRIIIA (ALIE mutant) or both hFcγRIIA and hFcγRIIIA (GASDALIE mutant). Only 20% of FcγR-humanized mice receiving ALIE mutant anti-hCD20 mAb survived re-challenge with EL4-hCD20 cells, while 77% of mice receiving GASDALIE mutant anti-hCD20 mAb survived re-challenge ($p < 0.0001$; Figure 6B). Further, only 36% ($p = 0.01$) of hFcγRIIIA/B-Tg mice (that lack all murine FcγRs and express only hFcγRIIIA and hFcγRIIIB) survive EL4-hCD20 re-challenge, again demonstrating that expression of hFcγRIIA is required for optimal induction of an anti-tumor vaccinal effect. Thus, engagement of hFcγRIIA, which is the only activating hFcγR expressed by human DCs, is required for the generation of an anti-tumor vaccinal effect by passively administered anti-tumor mAb.

DISCUSSION

It has long been hypothesized that passive administration of anti-tumor antibodies may generate immune complexes that, upon uptake by antigen-presenting cells, stimulate anti-tumor cellular immunity. Taken together, our results now mechanistically demonstrate how passively administered anti-tumor antibody achieves such an effect using a lymphoma cell line that expresses a model tumor neoantigen. Anti-tumor mAb opsonizes tumor cells and targets them for killing by FcγR-mediated ADCC, a process that generates antibody:tumor antigen immune complexes. These immune complexes engage activating FcγRs expressed by CD11c$^+$ antigen-presenting cells, which results in stimulation of DC maturation and presentation of tumor antigens to T cells, thereby leading to long-term anti-tumor cellular memory formation (Figure 7) (Boruchov et al., 2005; Dhodapkar et al., 2005; Kalergis and Ravetch, 2002; Nimmerjahn and Ravetch, 2008). In the human FcγR system, the vaccinal effect requires interactions with hFcγRIIA, the sole activating FcγR expressed by DCs. Thus, these results now mechanistically explain how passively administered anti-tumor mAb stimulates anti-tumor cellular immune responses in vivo and suggest novel methods for augmenting such an effect.

It is clear that activating FcγRs expressed by antigen-presenting cells, especially DCs, are capable of capturing antibody/tumor antigen immune complexes. Upon immune complex binding, DCs undergo maturation, upregulate MHC-II and co-stimulatory molecules, and stimulate CD4 and CD8 T cells responses through traditional antigen presentation and cross-presentation (Nimmerjahn and Ravetch, 2008; Rafiq et al., 2002). DCs loaded with antibody/tumor antigen immune complexes stimulate potent T cell responses that are capable of eradicating tumors (Kalergis and Ravetch, 2002). In vitro studies have demonstrated that anti-CD20 mAb treatment of lymphoma cells stimulates DC maturation and CD8 T cell activation (Selenko et al., 2002), and a synergistic effect between vaccination with hCD20$^+$ tumor cells and anti-hCD20 mAb treatment has been demonstrated in mice (Gadri et al., 2009). CD8$^+$ DCs are

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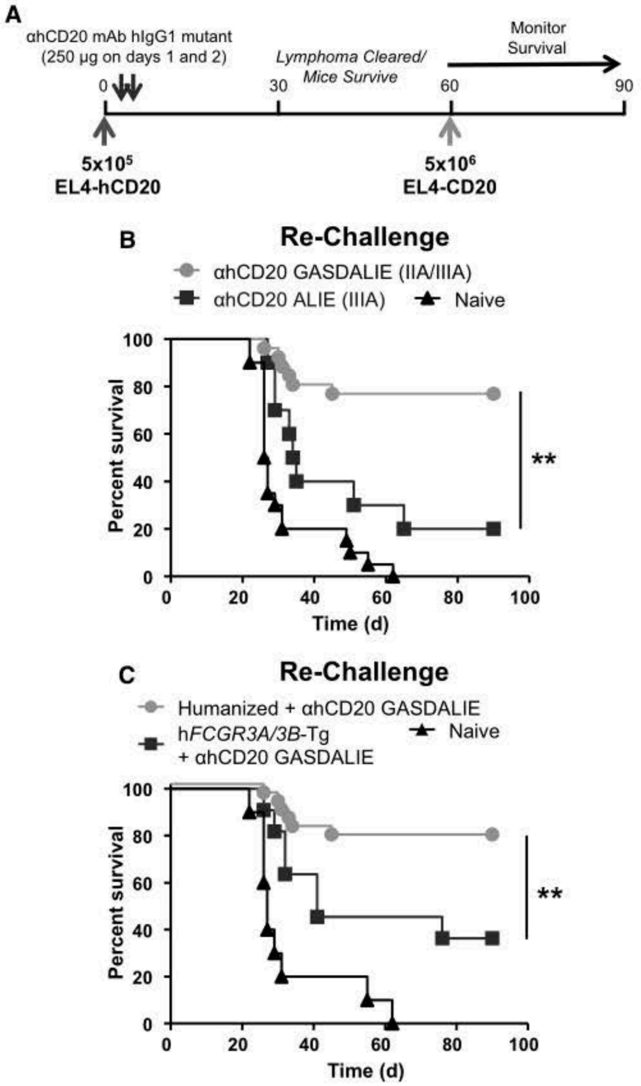

Figure 6. Selective Engagement of hFcγRIIA Mediates an Anti-tumor Vaccinal Effect

(A) Experimental protocol. FcγR-humanized mice were injected i.v. with 5×10^5 EL4-hCD20 lymphoma cells on day 0 (red arrow) and received 250 μg of hIgG1 mutant anti-hCD20 mAb on days 1 and 2 (blue arrows). On day 60, surviving mice were re-challenged i.v. with 5×10^6 EL4-hCD20 lymphoma cells (green arrow), and survival was monitored daily.

(B) Surviving tumor-primed mice that received GASDALIE hIgG1 anti-hCD20 mAb (green circles; n = 28) or ALIE hIgG1 anti-hCD20 mAb (blue squares; n = 10) from Figure 5B, or naive mice (black triangles; n = 15) were re-challenged with EL4-hCD20 cells.

(C) Surviving tumor-primed FcγR-humanized (green circles; n = 28) or hFcγRIIIA/B-Tg mice (blue squares, n = 11) that received GASDALIE hIgG1 anti-hCD20 mAb from Figure 5C, or naive mice (black triangles; n = 10) were re-challenged with EL4-hCD20 cells. Significant differences between groups are indicated: $^{**}p < 0.01$.

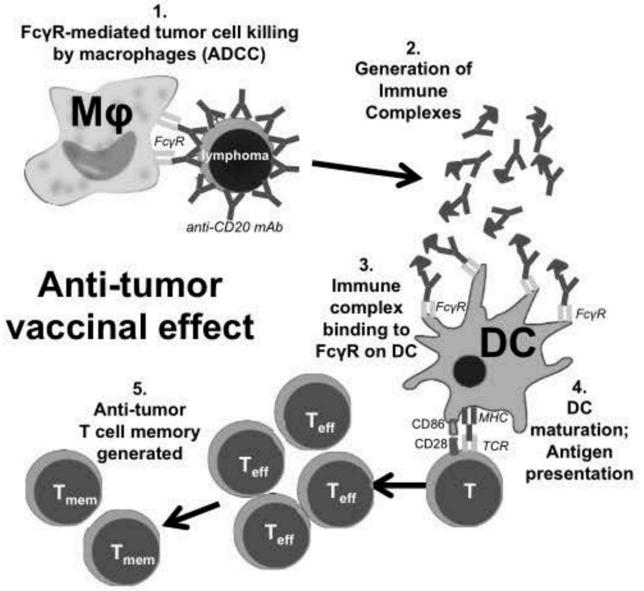

Figure 7. Model for the Generation of an Anti-tumor Vaccinal Effect

Anti-tumor mAb opsonizes tumor cells and targets them for killing by FcγR-mediated ADCC, a process that generates antibody:tumor antigen immune complexes. These immune complexes engage activating FcγRs expressed by mouse or human $CD11c^+$ cells, which results in stimulation of DC maturation and presentation of tumor antigens to T cells, thereby leading to long-term anti-tumor cellular memory formation.

considered to be excellent cross-presenters of cell-associated antigens (Mayer et al., 2014), and soluble immune complexes stimulate cross-presentation by DCs more potently than antigen alone (Berlyn et al., 2001; Rafiq et al., 2002). Correspondingly, a much larger fraction of $CD8^+$ spleen $CD11c^{hi}$ cells expresses mFcγRIV compared to $CD8^-CD11c^{hi}$ cells (60% versus 28%, Figure S2E), suggesting that $CD8^+$ DCs may play a significant role during the induction of the mAb-mediated anti-tumor vaccinal effect. Further, clinical trials have shown that combining anti-CD20 mAb treatment with administration of immunomodulatory cytokines that promote the activation of DCs or T cell responses, such as IFN-α (Kimby et al., 2008) or GM-CSF (Cartron et al., 2008), synergistically increased anti-CD20 mAb efficacy, suggesting that augmenting antigen presentation and T cell responses in the context of anti-tumor mAb therapy may augment an anti-tumor vaccinal effect. Murine studies have also demonstrated a heightened vaccinal effect when administration of the pleiotropic cytokine IL-2, which activates both innate cells and T cells, is combined with anti-hCD20 mAb treatment (Abès et al., 2010). Thus, our studies now mechanistically explain how the anti-tumor cellular immune responses generated by passive antibody treatment are generated in vivo.

While healthy individuals are normally tolerized to self-antigens such as CD20 and would not develop memory T cells reactive with self-antigens, cancer-bearing patients often break tolerance and autoimmune disorders are common in these patients (Abu-Shakra et al., 2001). Thus, while tolerance to overexpressed antigens, oncoproteins, tumor suppressor proteins, differentiation antigens, or neoantigens engaged by antibodies may be broken and lead to the generation of anti-tumor memory T cells, these cells are often anergized or exhausted and unable to mount an effective cytotoxic T cell response to the tumor. Activating these T cells to become effector cells and target tumor cells is thus a goal of

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current immunotherapy approaches, most recently achieved by blocking inhibitory signals such as CTLA-4 (Hodi et al., 2010) and PD-1 (Brahmer et al., 2012; Topalian et al., 2012). Our data support an alternative approach in which combining anti-tumor cytotoxic antibody therapeutics with various immunotherapies that boost cellular immune responses (i.e., agonistic anti-CD40 mAb) (Li and Ravetch, 2011), or antagonistic anti-CTLA-4 or anti-PD-1 mAbs) may synergistically combine with an anti-tumor mAb vaccinal effect to boost cellular memory formation. Thus, our results suggest a general mechanism by which anti-tumor antibodies can stimulate anti-tumor cellular immune responses against a variety of tumor antigens.

Significant efforts were put toward identifying the antigen-specific T cells that mediate the vaccinal effect in this study, but ex vivo re-stimulation (ELISPOT and intracellular cytokine staining) studies with tumor cell lysates, peptides, or irradiated tumor cells were not sensitive enough to detect rare tumor-specific T cells. Regardless, conclusions regarding the specificity of the anti-tumor vaccinal effect T cell response can be made. Mice primed with mAb and hCD20$^+$ EL4 lymphoma cells rejected lymphoma re-challenge with hCD20$^+$ EL4 cells but not wild-type EL4 cells (Figure 1C). This result suggests that the vaccinal effect T cell response is directed at the hCD20 neoantigen and that no detectable epitope-spreading occurs in this model. We have confirmed that the T cell response is, at least in part, directed at hCD20 because mice primed with mAb and hCD20$^+$ EL4 lymphoma rejected re-challenge with a distinct tumor cell line expressing hCD20, but not the same cell line expressing a control antigen (Figure 1D). Whether all vaccinal effect anti-tumor T cell responses are solely directed at the mAb-targeted antigen, as in the current EL4 tumor model, or whether different tumor models or different tumor microenvironments (i.e., lymphoid versus solid tumors) will result in different mAb-induced anti-tumor T cell responses and epitope spreading remains unclear. Whether combining passive anti-tumor mAb with checkpoint inhibitor blockade or adjuvants will result in synergistically enhanced epitope spreading and anti-neoantigen T cell responses is also unknown.

Our studies in FcγR-humanized mice with hIgG1 anti-hCD20 mAb-mediated ADCC of hCD20$^+$ tumor cells clearly demonstrate that hFcγRIIIA is both necessary and sufficient for ADCC-mediated clearance of antibody-coated tumor cells; hFcγRIIA plays no role in this process (Figure 5). These results correspond to findings in humans that *FCGR3A* polymorphisms correlate with response rates in lymphoma patients treated with anti-CD20 mAb (Cartron et al., 2002) or breast cancer patients treated with anti-Her2 mAb (Musolino et al., 2008). Importantly, FcγR signaling is required for ADCC in vivo, rather than simple cross-linking of antigen (de Haij et al., 2010). Because they solely express hFcγRIIIA, dogma has dictated that NK cells are the main mediators of ADCC in humans (Seidel et al., 2013). Further promoting the belief that NK cells are the major mediators of ADCC in humans, NK cells from human peripheral blood are routinely used during in vitro ADCC assays, which inadequately attempt to artificially re-create a complex in vivo process. Nonetheless, it has been demonstrated that CLOD-sensitive macrophages (Uchida et al., 2004) (Figure S7A), but not NK cells, are required for ADCC by mIgG in the context of murine FcγRs. This was partially thought to be due to the lone expression of FcγRIII on murine NK cells (Otten et al., 2008), which weakly interacts with mouse antibody Fc compared to FcγRIV. Therefore, we have now clearly determined the cellular requirements for hIgG1-triggered ADCC mediated by human FcγRs and demonstrate that CLOD-sensitive macrophages mediate ADCC of antibody-coated target cells in vivo in the context of hIgG1 antibody and the human FcγR system (Figures S7B and S7C). This result is significant, because new therapies aimed at augmenting human NK cell activity in vivo to enhance ADCC of mAb-targeted tumor cells are currently under investigation. Further studies determining any functional differences between murine and human NK cells will shed more light on this important matter.

The results reported here highlight the importance of properly engineering antibody therapeutics to engage the appropriate FcγRs to mediate appropriate effector functions. Current efforts to augment anti-tumor antibodies have only focused on enhancing their cytotoxic effects by modulating hIgG1 Fc interactions with hFcγRIIIA to augment ADCC by innate cells, as exemplified by the next-generation glyco-engineered anti-hCD20 mAb, obinutuzumab (Goede et al., 2014). Obinutuzumab is afucosylated for augmented affinity to only hFcγRIIIA, and accordingly, extends survival by ~1 year in CLL patients when directly compared to an unmodified anti-CD20 antibody (Rituximab). However, afucosylation of obinutuzumab does not affect Fc engagement of hFcγRIIA, which is the sole activating hFcγR expressed on human antigen-presenting DCs for engagement of immune complexes and stimulation of T cell responses. Thus, our current results argue that an ideal anti-tumor therapeutic should not only optimally engage hFcγRIIIA for cytotoxic effector function, but also hFcγRIIA on DCs in order to induce long-term cellular anti-tumor immunity.

EXPERIMENTAL PROCEDURES

Cell Lines and Mice

EL4-WT and 293T cells were obtained from ATCC and maintained in Dulbecco's minimum essential medium (DMEM; Life Technologies) supplemented with 10% fetal bovine serum (Life Technologies), 100 U/ml of penicillin, and 100 μg/ml of streptomycin (Life Technologies). EL4-hCD20 cells were obtained from Oliver Press (Fred Hutchinson Cancer Research Center, Seattle, WA) with permission from Josée Golay (Ospedali Riuniti di Bergamo, Bergamo, Italy) and maintained in RPMI-1640 medium (Life Technologies) supplemented with 10% fetal bovine serum, 100 U/ml of penicillin, and 100 μg/ml of streptomycin. B6BL cells, a spontaneous B cell lymphoma line isolated from p53$^{fl/fl}$CD19-Cre$^+$ mice on pure B6 genetic background, have been previously described (Robbiani et al., 2009) and were retrovirally transduced with constructs encoding either hCD20 or mCD20 (pVPack Vectors, Agilent Technologies), selected to make stable cell lines, and sorted for CD20$^+$ cells, as described previously (Li and Ravetch, 2011). C57BL/6 mice were purchased from Jackson Laboratories. *Fcer1g*$^{-/-}$ (Takai et al., 1994), *Fcgr4*$^{-/-}$ (Nimmerjahn et al., 2010), FcRα null (Smith et al., 2012), *FCGR3A/B*-Tg (Li et al., 1996) (crossed to FcRα null mice), and *FCGR2A*-Tg (McKenzie et al., 1999) (crossed to *Fcer1g*$^{-/-}$*Fcgr2b*$^{-/-}$ mice) mice on the C57BL/6 genetic background have been previously described. FcγR-humanized mice, which express all hFcγRs on the FcRα null C57BL/6 genetic background, have been described (Smith et al., 2012). *Fcgr4*^{flox} mice (Nimmerjahn et al., 2010) were crossed with mice expressing cre recombinase under the control of CD11c promoter/enhancer regions (B6.Cg-Tg(Itgax-cre)1-1Reiz/J mice, Jackson Laboratories). hCD20-Tg mice were kindly provided by Dr. Andrew Chan (Genentech) and crossed onto the FcγR-humanized background. All mice were maintained in a specific pathogen-free facility at the Rockefeller University, and all studies were approved by the Rockefeller University Institutional Animal Care and Use Committee.

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Antibodies, Flow Cytometry, and Other Reagents

To generate CAT-13.6E12 and 2B8 mAb constructs, total RNA was obtained from hybridoma cells (DSMZ and ATCC, respectively), and cDNA was generated by using SuperscriptIII reverse transcriptase (Life Technologies) and immunoglobulin gene-specific primers. The V_H and V_K genes were amplified by PCR and cloned in frame into mammalian expression vectors with mouse IgG2a, mouse IgG1, mouse DA265 mutant, mouse Kappa, hIgG1, or huKappa Fc backbones. The human G236A, A330L/I332E, and GASDALIE Fc mutants were generated by site-directed mutagenesis with PCR amplification of the entire vector, using complementary primers containing the desired point mutations, as described (Li and Ravetch, 2011). Antibodies were produced by transient transfection of 293T cells and subsequent protein G purification from culture supernatants, as described (Nimmerjahn et al., 2005). Anti-mCD4 antibody (clone GK1.5) with a hIgG1 backbone and containing GASDALIE point mutations was generated previously (Smith et al., 2012). Fluorescently conjugated antibodies and staining procedures are listed in the Supplemental Experimental Procedures. To deplete macrophages in vivo, mice received 200 μl of clodronate liposomes or PBS liposomes intravenously (i.v.) through lateral tail veins (Clophosome-A, Formumax).

Tumor Model

For the primary tumor challenge, mice were injected i.v. through lateral tail veins with 5×10^5 EL4-hCD20 cells in 200 μl PBS on day 0. Mice then received intraperitoneal (i.p.) injections of 100 μg of antibody in 200 μl of PBS on days 1, 4, 7, 10, and 13. Survival was assessed daily. In some experiments, 90 days after primary tumor challenge, surviving mice were re-challenged i.v. with 5×10^6 EL4-hCD20 or EL4-WT cells, with survival assessed daily. In experiments in which hIgG1 antibodies or mutants were administered, FcγR-humanized mice were given 5×10^5 EL4-hCD20 cells in 200 μl PBS on day 0, with 250 μg of antibody in 200 μl of PBS given i.p. on days 1 and 2. Surviving mice were re-challenged with 5×10^6 EL4-hCD20 cells on day 60. In some experiments, mice were re-challenged i.v. with 5×10^4 B6BL cells expressing either hCD20 or mCD20.

In adoptive transfer experiments, splenocytes from mice 30 days after primary tumor challenge were harvested and red blood cells were lysed. In some cases, $CD3^+$ cells were negatively selected using magnetic beads (Miltenyi Biotec). Then, 50×10^6 total splenocytes or 15×10^6 $CD3^+$ cells were adoptively transferred into naive C57BL/6 mice. One day later, the mice were challenged with 5×10^5 EL4-hCD20 cells in 200 μl PBS, with survival assessed daily.

Statistics

Statistical differences between survival rates were analyzed by comparing Kaplan-Meier curves using the log-rank test and GraphPad Prism Software. All other statistical differences were compared using the Student's t test analysis.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, seven figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2015.04.016.

AUTHOR CONTRIBUTIONS

D.J.D. and J.V.R. designed experiments, analyzed the data, and wrote the manuscript. D.J.D. performed the experiments.

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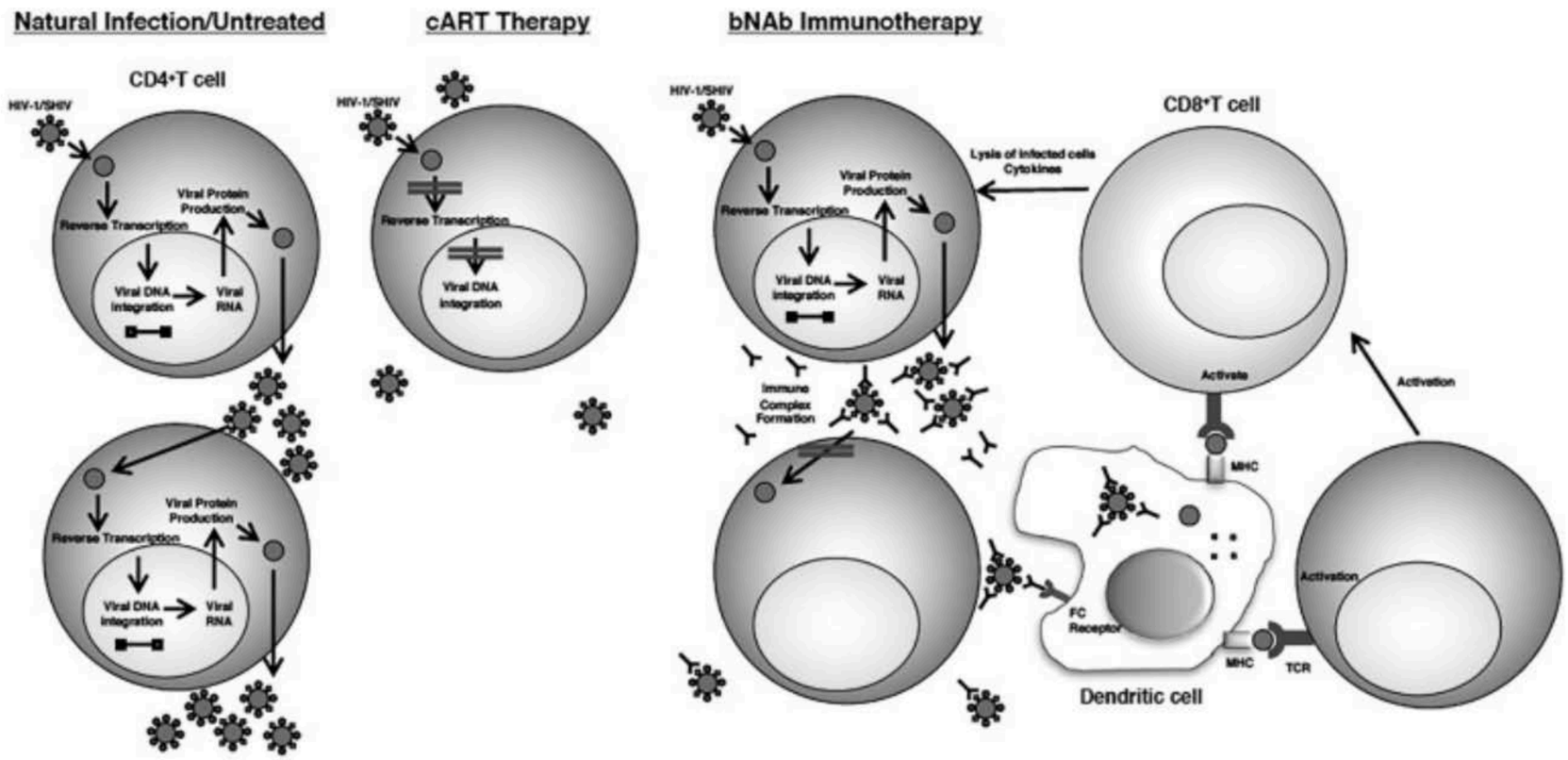
Anti-HIV bNAb treatment induces CD8 Memory in SHIV infected macaques



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M. Martin, 2017

From: Schwetz, Tara (NIH/NIAID) [E]
Sent: Tue, 1 May 2018 12:15:50 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Paules, Catharine (NIH/NIAID) [E];Folkers, Greg (NIH/NIAID) [E];Conrad, Patricia (NIH/NIAID) [E]
Subject: Re: Gates and POTUS

Will do. I just heard from Martin, so I will clean this up and send to Larry shortly.

Best,

Tara A. Schwetz, PhD
NIH/NIAID
(b)(6)

Sent from my iPhone

On May 1, 2018, at 7:55 AM, Fauci, Anthony (NIH/NIAID) [E] (b)(6) wrote:

Tara:
Nice job. I have made a few changes. Also, confirm the total amount of money (now at (b)(5) and change that when you get the correct amount. After you do that, please send to Larry. I will be at the Fogarty event.
Thanks,
tony

From: Schwetz, Tara (NIH/NIAID) [E]
Sent: Monday, April 30, 2018 10:40 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Paules, Catharine (NIH/NIAID) [E] (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: Fwd: Gates and POTUS

Tony,

Please find attached the draft document/briefer on flu for Secretary Azar. Note: we are double checking the budget number highlighted.

Please do let us know if you need anything else.

Best,

Tara A. Schwetz, PhD
NIH/NIAID
(b)(6)

AMERICAN OVERSIGHT

Sent from my iPhone

Begin forwarded message:

On Apr 30, 2018, at 6:57 PM, Fauci, Anthony (NIH/NIAID) [E] (b)(6) wrote:

(b)(6)

Sent from my iPad

Begin forwarded message:

From: "Tabak, Lawrence (NIH/OD) [E]" (b)(6)
Date: April 30, 2018 at 6:02:54 PM EDT
To: "Fauci, Anthony (NIH/NIAID) [E]" (b)(6)
Cc: "Collins, Francis (NIH/OD) [E]" (b)(6)
Subject: Gates and POTUS

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The Secretary took note of the lack of awareness about the universal flu vaccine effort at the WH. The Secretary will be meeting with POTUS on Wednesday and wants to be armed with the relevant bullet points of what we are doing (including the amount of money being spent). I could use the "1/2" pager by tomorrow morning please so the Secretary can make the "pitch" to the POTUS. Francis will be seeing the Secretary later in the afternoon tomorrow and he can reinforce.
Thanks,
Larry

--

<2018-04-30_NIH Flu Vaccine Efforts_v1 - with Fauci tracked changes.docx>

AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Tue, 1 May 2018 11:55:47 +0000
To: Schwetz, Tara (NIH/NIAID) [E]
Cc: Paules, Catharine (NIH/NIAID) [E];Folkers, Greg (NIH/NIAID) [E];Conrad, Patricia (NIH/NIAID) [E]
Subject: RE: Gates and POTUS
Attachments: 2018-04-30_NIH Flu Vaccine Efforts_v1 - with Fauci tracked changes.docx

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Cc: Paules, Catharine (NIH/NIAID) [E] (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
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(b)(6)

Sent from my iPad

Begin forwarded message:

AMERICAN OVERSIGHT

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Cc: "Collins, Francis (NIH/OD) [E]" (b)(6)
Subject: Gates and POTUS

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Larry

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AMERICAN OVERSIGHT

Page 1124 of 2068

Withheld pursuant to exemption

(b)(5)

of the Freedom of Information and Privacy Act

AMERICAN OVERSIGHT

From: Billet, Courtney (NIH/NIAID) [E]
Sent: Tue, 1 May 2018 11:03:56 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Folkers, Greg (NIH/NIAID) [E];Conrad, Patricia (NIH/NIAID) [E];Stover, Kathy (NIH/NIAID) [E]
Subject: Re: For your review. PR on launch of Phase 2 universal flu vaccine study

Yes of course, that's the plan!

Sent from my iPhone

On Apr 30, 2018, at 8:12 PM, Fauci, Anthony (NIH/NIAID) [E] (b)(6) wrote:

Looks fine. Any way that we can reach out a bit more than usual to the press. This is a big deal – starting a phaser 2 trial on a universal influenza vaccine

---

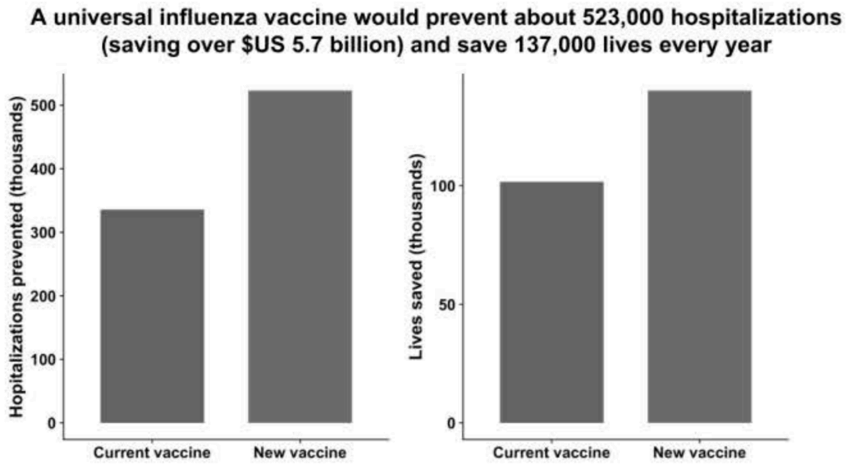
From: Billet, Courtney (NIH/NIAID) [E]
Sent: Monday, April 30, 2018 3:52 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Folkers, Greg (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6) Stover, Kathy (NIH/NIAID) [E] (b)(6)
Subject: ASF: For your review. PR on launch of Phase 2 universal flu vaccine study

Attached is our draft press release on the launch of a Phase 2 study of the BiondVax universal flu vaccine. There is a quote for you as follows:

"The 2017-2018 influenza season in the United States was among the worst of the last decade and serves as a reminder of the urgent need for a more effective and broadly protective influenza vaccine," said NIAID Director Anthony S. Fauci, M.D. "An effective universal influenza vaccine would lessen the public health burden of influenza, alleviate suffering and save lives. There are numerous paths of inquiry that the scientific community is pursuing, with each new study yielding more critical information and bringing us closer to our shared goal."

AMERICAN OVERSIGHT

From: Alison Galvani
Sent: Tue, 1 May 2018 00:56:19 -0400
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Sah, Pratha;Meagan Fitzpatrick
Subject: Please disregard previous figs - Revised fig attached for entire US & in terms of savings
Attachments: Projected Universal Flu Benefits for Entire US.pdf



New figure for the entire US. This figure includes economic benefits and presents results in terms of savings (rather than reduced losses). Now very simple, intuitive and positive.

--
Alison Galvani, PhD

Director, Yale Center for Infectious Disease Modeling and Analysis (CIDMA)
Burnett and Stender Families Professor of Epidemiology
Yale School of Public Health
Yale School of Medicine
New Haven, CT 06520

(b)(6)
http://cidma.yale.edu/

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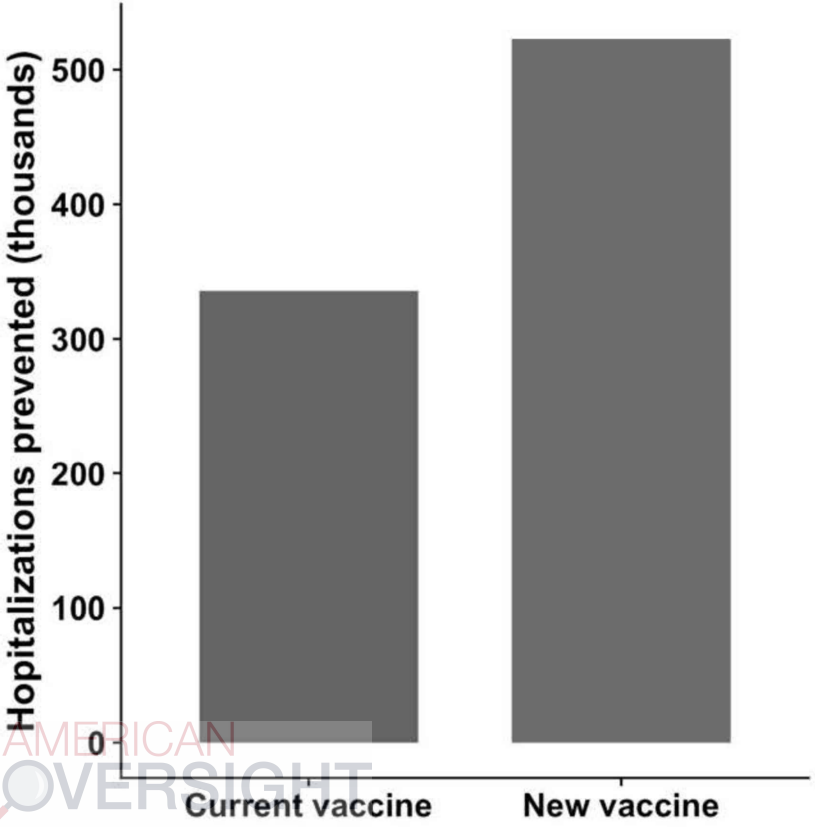
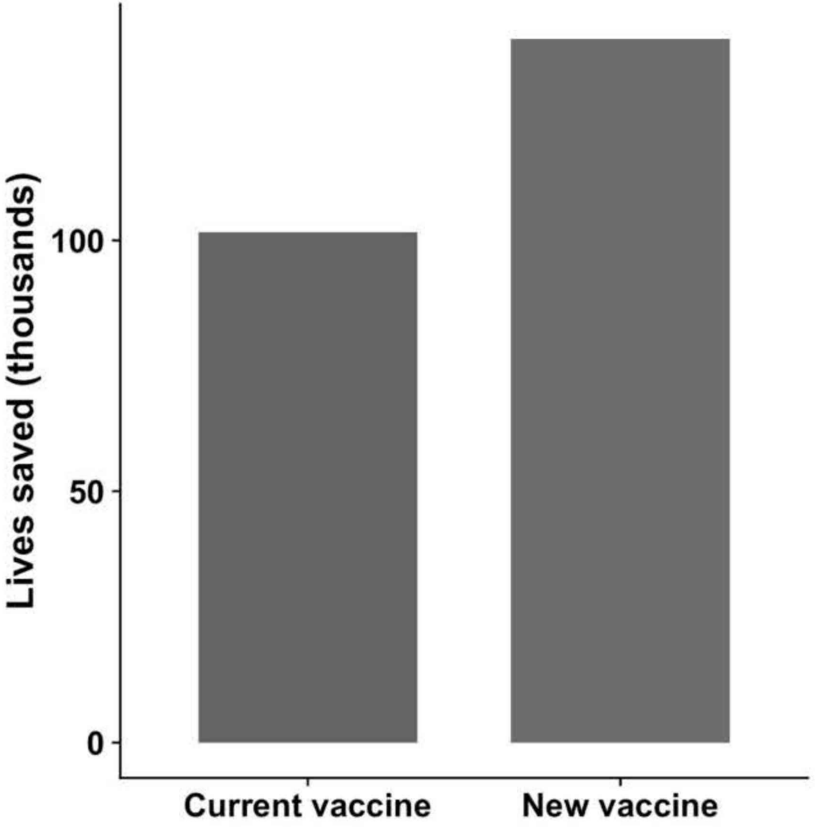
A universal influenza vaccine would prevent about 523,000 hospitalizations (saving over $US 5.7 billion) and save 137,000 lives every year





AMERICAN OVERSIGHT

From: Schwetz, Tara (NIH/NIAID) [E]
Sent: Tue, 1 May 2018 02:40:06 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Paules, Catharine (NIH/NIAID) [E];Folkers, Greg (NIH/NIAID) [E];Conrad, Patricia (NIH/NIAID) [E]
Subject: Fwd: Gates and POTUS
Attachments: 2018-04-30_NIH Flu Vaccine Efforts_v1.docx, ATT00001.htm

Tony,

Please find attached the draft document/briefer on flu for Secretary Azar. Note: we are double checking the budget number highlighted.

Please do let us know if you need anything else.

Best,

Tara A. Schwetz, PhD
NIH/NIAID
(b)(6)

Sent from my iPhone

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(b)(6)

Sent from my iPad

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Cc: "Collins, Francis (NIH/OD) [E]" (b)(6)
Subject: Gates and POTUS

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AMERICAN OVERSIGHT

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Thanks,

Larry

--

AMERICAN OVERSIGHT

Page 1130 of 2068

Withheld pursuant to exemption

(b)(5)

of the Freedom of Information and Privacy Act

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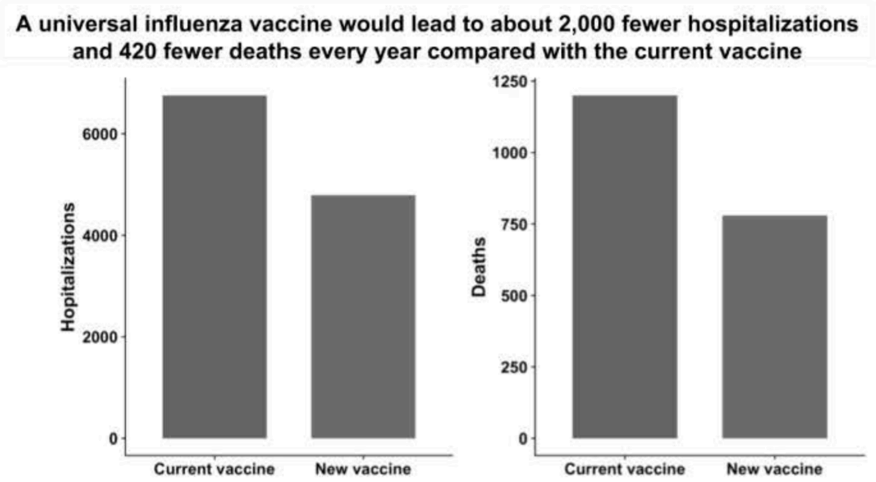
AMERICAN
OVERSIGHT

From: Alison Galvani
Sent: Mon, 30 Apr 2018 22:33:09 -0400
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Sah, Pratha;Meagan Fitzpatrick
Subject: Very simple figure showing reduction in hospitalizations and deaths
Attachments: Projected Universal Flu Benefits (1).pdf



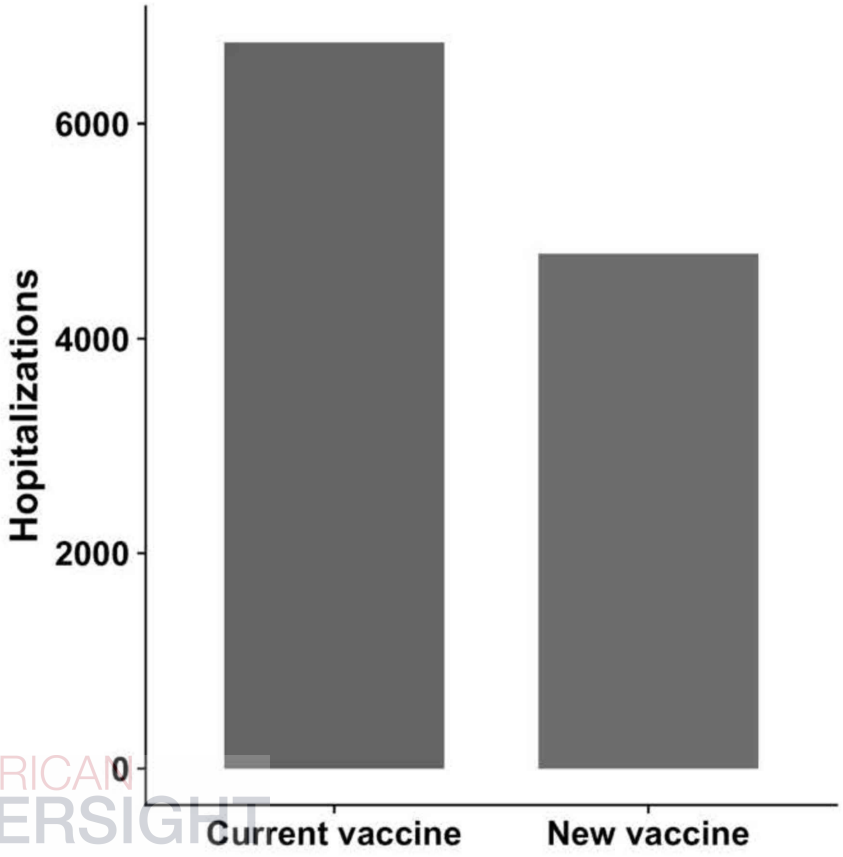
--
Alison Galvani, PhD

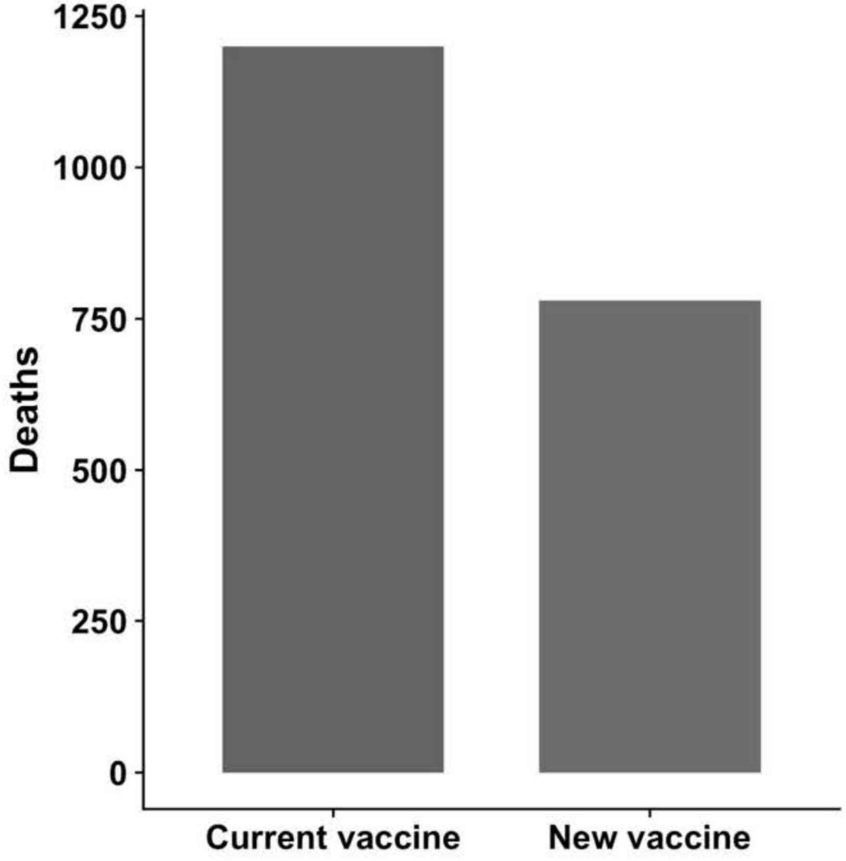
Director, Yale Center for Infectious Disease Modeling and Analysis (CIDMA)
Burnett and Stender Families Professor of Epidemiology
Yale School of Public Health
Yale School of Medicine
New Haven, CT 06520

(b)(6)
http://cidma.yale.edu/

AMERICAN OVERSIGHT

A universal influenza vaccine would lead to about 2,000 fewer hospitalizations and 420 fewer deaths every year compared with the current vaccine





AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Tue, 1 May 2018 01:51:41 +0000
To: Paules, Catharine (NIH/NIAID) [E]
Subject: RE: Gates and POTUS

OK. I will be there. I believe that you will not need to skip anything. Tara has precise instructions from me and we will review in the AM.

From: Paules, Catharine (NIH/NIAID) [E]
Sent: Monday, April 30, 2018 8:46 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: Re: Gates and POTUS

Sounds good. I have BLS training scheduled but can skip if need be. Will stop by your office around 730 and check in.

Sent from my iPhone

On Apr 30, 2018, at 8:33 PM, Fauci, Anthony (NIH/NIAID) [E] (b)(6) wrote:

Thanks. Tara's on it. We can all go over the ½ - pager tomorrow AM

From: Paules, Catharine (NIH/NIAID) [E]
Sent: Monday, April 30, 2018 8:31 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Schwetz, Tara (NIH/NIAID) [E] (b)(6)
Subject: Re: Gates and POTUS

Let me know if I can help. On my cell (b)(6)

Sent from my iPhone

On Apr 30, 2018, at 6:57 PM, Fauci, Anthony (NIH/NIAID) [E] (b)(6) wrote:

(b)(6)

Sent from my iPad

Begin forwarded message:

From: "Tabak, Lawrence (NIH/OD) [E]" (b)(6)
Date: April 30, 2018 at 6:02:54 PM EDT
To: "Fauci, Anthony (NIH/NIAID) [E]" (b)(6)
Cc: "Collins, Francis (NIH/OD) [E]" (b)(6)
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AMERICAN OVERSIGHT

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Thanks,
Larry

--

AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Tue, 1 May 2018 00:06:52 +0000
To: Eisinger, Robert (NIH/NIAID) [E]
Subject: FW: Fogarty panelist slides (pdfs), update re Sen Reed
Attachments: QAKarim.pdf, Bill Pape.pdf, Sten Vermund.pdf

Did you forward our PDFs to them?

From: Puderbaugh, Ann (NIH/FIC) [E]
Sent: Monday, April 30, 2018 3:14 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Eisinger, Robert (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: Fogarty panelist slides (pdfs), update re Sen Reed

Hello,

Regarding tomorrow's Fogarty@50 event, we have discovered that Natcher does not provide monitors so panelists can view slides during events. Given that, I thought you might like to see the pdfs of the ID panel presentations (Dr. Bekker's has not yet arrived).

Also, Sen. Jack Reed, who remembers Cong. Fogarty from his youth, will be joining us to say a few words as we reconvene from the lunch break at 12:30 p.m. Dr. Collins, who is escorting him, will then make his presentation, instead of at 2 p.m., as previously scheduled.

Please let me know if you have any questions.

Many thanks for your participation,
Ann

Ann Puderbaugh
Communications Director
Fogarty International Center
National Institutes of Health
31 Center Drive, Room B2C/29
Bethesda MD 20892-2220
Tel. (b)(6)
(b)(6)
www.fic.nih.gov

Connect with the **Fogarty International Center at NIH**:

Fogarty email subscriptions | @Fogarty_NIH | Fogarty on Facebook

AMERICAN OVERSIGHT

Ending AIDS as a public health threat:

Critical challenge of preventing HIV in young women in Africa

Fogarty 50th Anniversary Symposium - May 1, 2018

Quarraisha Abdool Karim

Associate Scientific Director: CAPRISA
Professor in Clinical Epidemiology, Columbia University
UNAIDS Special Ambassador for Adolescents and HIV

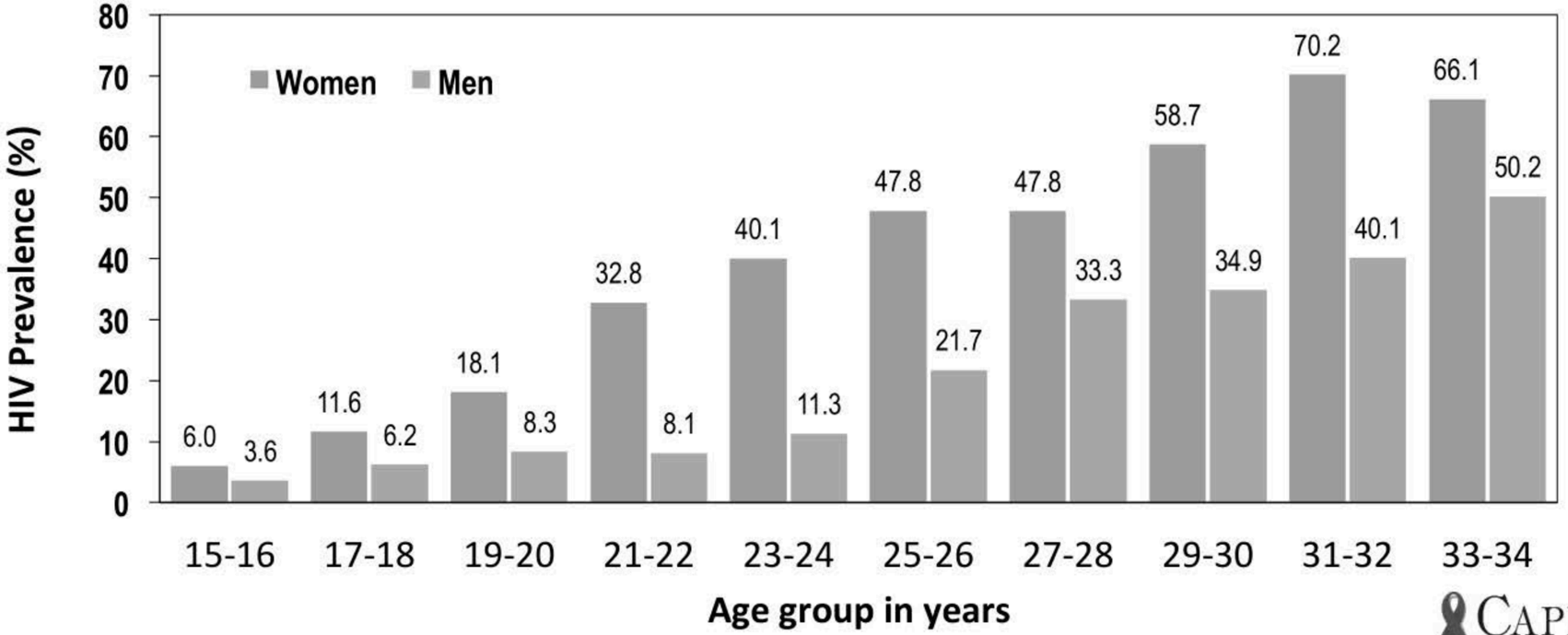
UNAIDS CAPRISA is the UNAIDS Collaborating Centre for HIV Research and Policy | NRF National Research Foundation CAPRISA hosts a DST-NRF Centre of Excellence in HIV Prevention | SAMRC CAPRISA hosts a MRC HIV-TB Pathogenesis and Treatment Research Unit | COLUMBIA UNIVERSITY | MAILMAN SCHOOL of PUBLIC HEALTH | UNIVERSITY OF KWAZULU-NATAL INYUVESI YAKWAZULU-NATALI

AMERICAN OVERSIGHT

In a series of papers in *Science*, *Nature Medicine* & *Lancet*, Fogarty trainees have revealed why young women are at high risk

Transmission networks and risk of HIV infection in KwaZulu-Natal, South Africa: a community-wide phylogenetic study

Tulio de Oliveira, Ayesha B M Kharsany*, Tiago Gräf, Cherie Cawood, David Khanyile, Anneke Grobler, Adrian Puren, Savathree Madurai, Cheryl Baxter, Quarraisha Abdool Karim, Salim S Abdool Karim*



CAPRISA

AMERICAN OVERSIGHT

"Cycle of HIV transmission" in Africa

Transmission networks and risk of HIV infection in KwaZulu-Natal, South Africa: a community-wide phylogenetic study

Tulio de Oliveira, Ayesha B M Kharsany*, Tiago Gräf, Cherie Cawood, David Khanyile, Anneke Grobler, Adrian Puren, Savathree Madurai, Cheryl Baxter, Quarraisha Abdool Karim, Salim S Abdool Karim*

Men
Mean age: 31.5 years (n=79)
Knew HIV status: 22%

*Community HIV prevalence in men aged 25-40 years: **40%*** (N=1548)*

Most young women <25 years acquire HIV from older men (Mean age difference = **8.7 years**, CI: 6.8-10.6)

39% of the men linked to a woman < 25 are simultaneously also linked to a woman 25-40 years

Most men & women 25-40 years acquire HIV from similarly aged partners (Mean age difference = **1.1 years**, CI: -0.6-2.8)

Young women <25 years
Knew HIV status: 23%
62% of male partners are 25-40 years

*Community HIV prevalence: **22%*** (N=2224)*

Women 25-40 years
Knew HIV status: 43%
63% of male partners are 25-40 years

*Community HIV prevalence: **60%*** (N=2680)*

When young women reach >25 years they continue the cycle

...but it's both behaviour and biology!

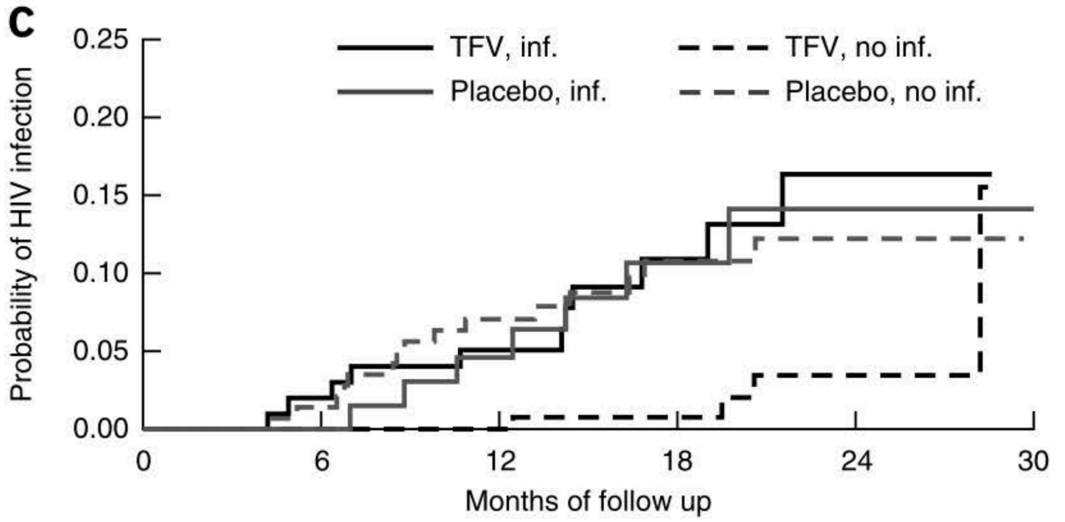
CAPRISA

AMERICAN OVERSIGHT

Genital inflammation increases risk of HIV & affects topical PrEP in women

Genital inflammation undermines the effectiveness of tenofovir gel in preventing HIV acquisition in women

Lyle R McKinnon[1–3,9], Lenine J Liebenberg[1,3,9], Nonhlanhla Yende-Zuma[1], Derseree Archary[1,3], Sinaye Ngcapu[1,3], Aida Sivro[1–3], Nico Nagelkerke[2], Jose Gerardo Garcia Lerma[4], Angela D Kashuba[5], Lindi Masson[1,6], Leila E Mansoor[1], Quarraisha Abdool Karim[1,7], Salim S Abdool Karim[1,7] & Jo-Ann S Passmore[1,6,8]



Genital inflammation (>5/9 cytokines) increased the risk of HIV acquisition

2.4 fold

(95% CI: 1.4 to 4.2)

p=0.002

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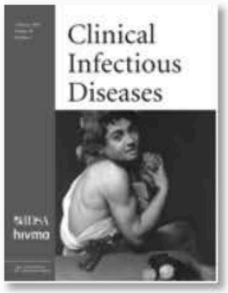
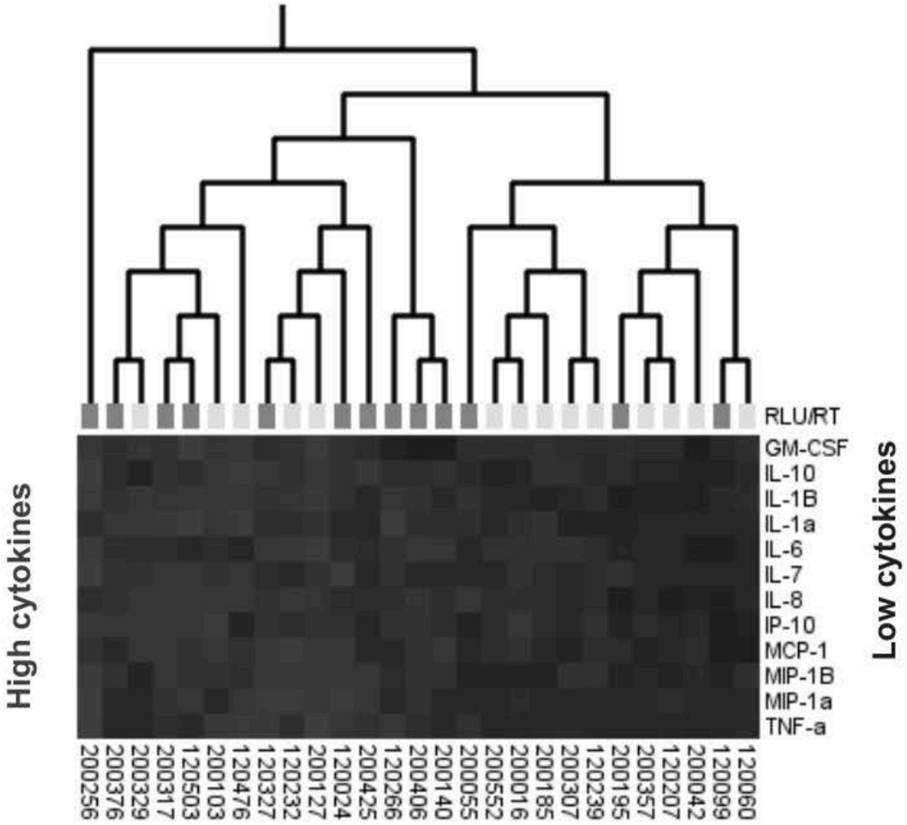
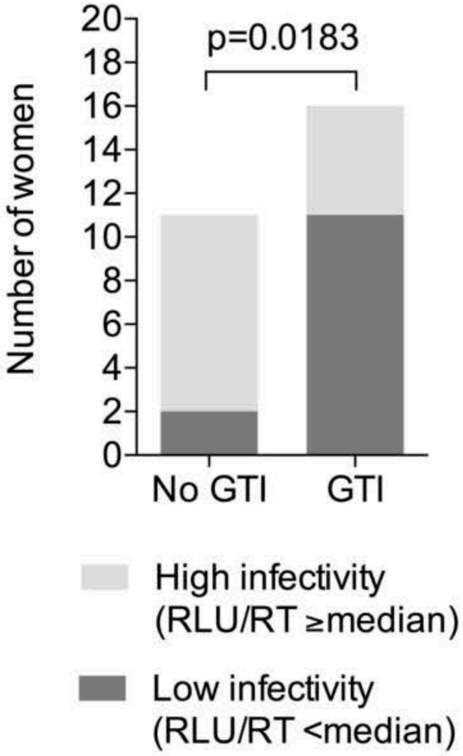


Cervicovaginal inflammation facilitates acquisition of less infectious HIV variants

Philippe Selhorst1,*, Lindi Masson1,2,*, Sherazaan D. Ismail1, Natasha Samsunder2, Nigel Garrett2, Leila E. Mansoor2, Quarraisha Abdool Karim2,3, Salim S. Abdool Karim2,3, Jo-Ann S. Passmore1,2,4,+, Carolyn Williamson1,+,4





Only 2/11 (18%) women who did not have pre-infection genital inflammation were infected by viruses with low infectivity (RLU/RT <median), compared to 11/16 (69%) women with inflammation

Viral infectivity was calculated as Relative Light Units (RLUs) generated per picogram Reverse Transcriptase (RT) activity in each stock as measured by Roche's colorimetric RT assay (Switzerland)

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What is causing genital inflammation and vulnerability to HIV in women?

	P. bivia+ OR*	P value
HC	19.2 (95% CI: 4.0-92.4)	p<0.001
HIV+	12.7 (95% CI: 2.1-77.8)	p=0.006

**adjusted odds ratio*

22 women were HIV positive & had inflammation – 9/22 (41%) had *P. Bivia* in the vaginal microbiome

Women with *P. bivia* were **19 times** more likely to have genital inflammation and **13 times** more likely to acquire HIV

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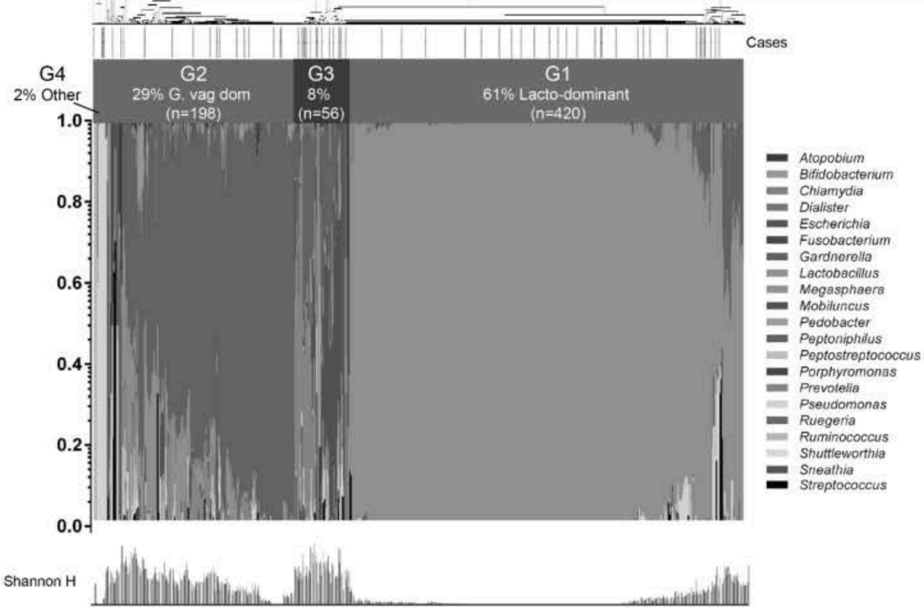
AMERICAN OVERSIGHT

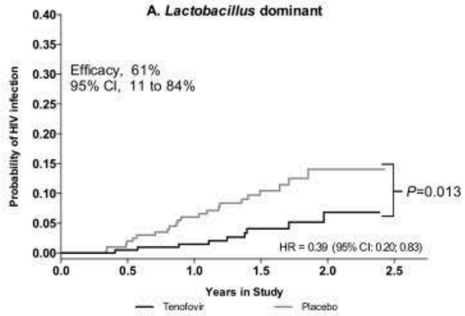
The vaginal microbiome: key factor in HIV risk and topical PrEP efficacy

Vaginal bacteria modify HIV tenofovir microbicide efficacy in African women

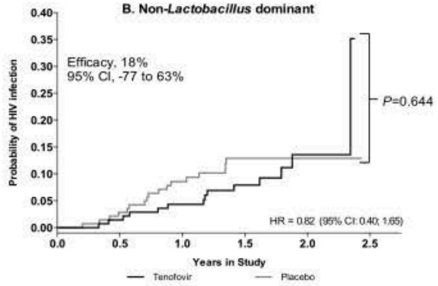
Nichole R. Klatt,[1*†] Ryan Cheu,[1‡] Kenzie Birse,[2,3‡] Alexander S. Zevin,[1‡] Michelle Perner,[2,3‡] Laura Noël-Romas,[2,3‡] Anneke Grobler,[4] Garrett Westmacott,[5] Irene Y. Xie,[2,3] Jennifer Butler,[2,3] Leila Mansoor,[4] Lyle R. McKinnon,[3,4] Jo-Ann S. Passmore,[6,4] Quarraisha Abdool Karim,[4,7] Salim S. Abdool Karim,[4,7] Adam D. Burgener[2,3,8*†]



Overall diversity plot of all women



Women with *Lactobacillus* dominance



Women with <50% *Lactobacilli*

CAPRISA

AMERICAN OVERSIGHT

Thanks to:

National Institutes of Health
Fogarty International Center

FOGARTY

CAPRISA

CENTRE FOR THE AIDS PROGRAMME OF RESEARCH IN SOUTH AFRICA

AMERICAN OVERSIGHT

AIDS accomplishments and Research needs to end AIDS in Latin America/Caribbean

Jean William Pape MD

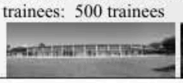
Howard and Carol Holtzmann Professor of Clinical Medicine, Center for Global Health, Division of Infectious Diseases, Department of Medicine, Weill Cornell Medical College, New York, New York, USA

Director,
Les Centres GHESKIO, Port-au-Prince, Haiti.

Fogarty 50th Anniversary Symposium, NIH Campus, Bethesda, Maryland
May 1, 2018

Les Centres GHESKIO (May 2, 1982)

- HIVAIDS/STI, TB Comprehensive Center for:
  - Translational research: 2 publications/month
  - Training: largest post graduate training center in Haiti
  - Services: one of the largest AIDS and TB centers in the Americas
- Extended to diarrheal diseases, malaria, dengue, HPV, nutrition…
- Post-earthquake: global health aimed at the poor: care for IDP, primary, vocational schools and microcredit programs
- Partnership: Ministry of Health and 116 local institutions
- Granted status of "Public Utility " by Haitian government (2000)
- Dedicated private sector foundation: FHAME
- Strong international collaboration: Cornell, F. Mérieux…
- Continuous support from NIH (1983), Fogarty (1988): 4th generations of Fogarty trainees: 500 trainees



Capacity Building by FIC

- 1989-1990: I was a Fogarty trainee and 1990-2018: FIC grantee : AITRP, ICOHRTA, Framework, training grants for HPV/cervical cancer and MDRTB led to the training of 494 Haitians at the MDs, PhDs, Masters level, essential to:
  1) Conduct translational research on OIs, TB, AIDS care/RX leading to development of HIV prevention and care models
  2) Train >16,000 health workers (public, private sectors)
  3) Scale-up GHESKIO HIV, STI, TB, cholera at the national level
  4) Establish public health programs with local institutions
     - Laboratory with the Ministry of Health Public Health Lab
     - 1st MPH and NP with local institution (Quisqueya University)
  5) Collaborative research network within the Americas:
     - ACTG, IMPAACT, GABRIEL
     - Trans Caribbean HIV/AIDS Network Initiative (TCHARI)
     - Caribbean Central America South A. AIDS network (CCASA*net*)
  6) Important support with NIAID after major 2010 earthquake

AMERICAN OVERSIGHT

HIV/AIDS IN LATIN AMERICA THE CARIBBEAN (LAC) REGIONAL OVERVIEW

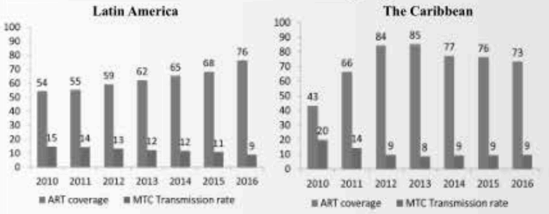
Latin America and Caribbean (2016)

- 2.1 million people living with HIV
- 0.5% adult HIV prevalence
- 115,000 new HIV infections
- 45,400 AIDS-related deaths
- 56% adults on antiretroviral treatment
- 53% children on antiretroviral treatment

Source: UNAIDS Data 2017

- **Elimination of transmission:** Complete cessation of incidence in a geographically defined area. Requires ongoing interventions to maintain.
  - Can we prevent HIV vertical transmission (PMTCT), transmission by blood/blood products and sexual transmission?
- **Elimination as a public health problem:** Reduction of incidence and morbidity below a specific (globally defined) level.
  - Can we reduce HIV incidence, mortality/morbidity to an accepted level?

Pregnant Women on Antiretroviral Treatment to Prevent Mother to Child Transmission (%), 2010-2016



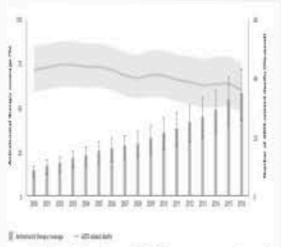
Countries with WHO Validation of the Elimination of MTCT of HIV/ syphilis

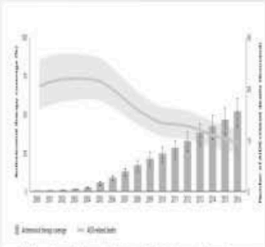
Cuba	Cayman Islands
Anguilla	Montserrat
St Kitts and Nevis	Antigua and Barbuda
Bermuda	Cayman Islands

Pan American Health Organization World Health Organization Source: UNAIDS Spectrum estimates 2017; Note: Estimates are rounded

ART Coverage and AIDS-related deaths in LAC

Latin America	The Caribbean
12% reduction of AIDS-related mortality	45% reduction of AIDS-related mortality





2016: 1/3 of new HIV diagnoses are in patients with a baseline CD4 of <200 mm3/mL

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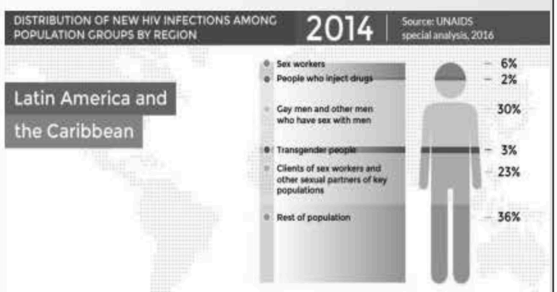
Characteristics of new HIV infections in LAC

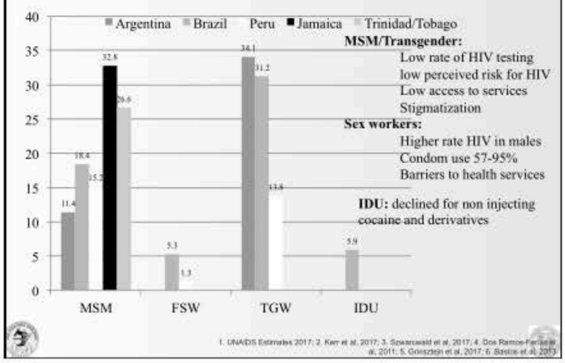


64% of new HIV infections are in key populations and their clients

15-24 y/o:1/3 new infections in the Caribbean and LA

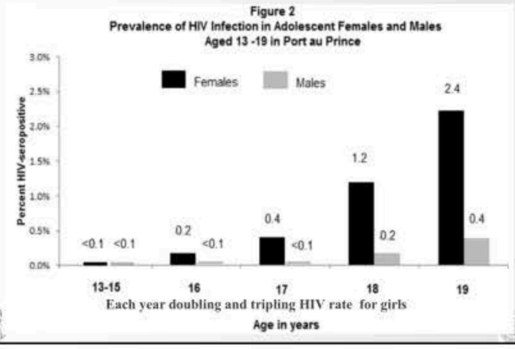
From www.avert.org

HIV Prevalence in Key Populations in LAC



1. UNAIDS Estimates 2017; 2. Kerr et al. 2017; 3. Szwarcwald et al. 2017; 4. Dos Ramos-Farias et al. 2011; 5. Grinsztejn et al. 2017; 6. Bastos et al. 2015

HIV Prevalence in Adolescents in Haiti



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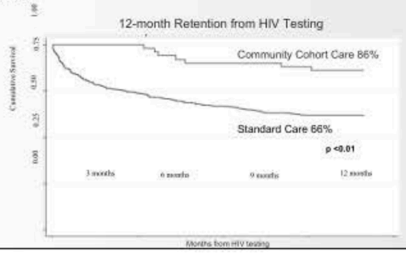
Community-Based HIV and Health Testing for High-Risk Adolescents and Youth

98% acceptability; recruit high risk ado: 2.6% HIV rate vs. 0.8% with previous method

- Monthly cohort session 6-8 vs monthly individual clinic visit: help social isolation
- All services provided in group setting in the community instead of the clinic
- All services in cohort group session with 1 nurse instead of sequential health care providers



Percentage of LAC countries with public policies for provision of the HIV prevention services by key population and selected services

Service or intervention	MSM (%)	FSW (%)	Transgender women (%)
HIV testing and counseling	100	97	94
STI diagnosis and treatment	90	91	84
PrEP	6	6	6
PEP	39	39	39
Condoms	100	96	81
Lubricants	89	89	83
Antiretroviral treatment (ART) for all	45	45	45
Peer-led community outreach activities	89	86	83
Sexual health information and education	100	96	91

Source: *PAHO. Country responses to the HIV Prevention Survey, May 2017.*; **Note:** *Percentages based on the response of 31 or 33 countries as of July 2017.*

Summary: What is needed to end AIDS in LAC?

- Have the tools to end AIDS as a public threat by 2030 if we have political and financial commitment for both care and prevention and use implementation science to focus on:
  - Key populations: MSM/TRANS, sex workers/clients with community-centered approach to increase HIV diagnoses and implement test/treat Prep, PEP, circumcision, STI control, condoms
  - Adolescents/young adults through comprehensive services
- Community approach with test/treat to prevent low CD4 late comers
- Address poverty, food insecurity, drivers of epidemic in many places
- In Haiti:
  - HIV prevalence: 2.0%; >97,000 on ART (30% in the GHESKIO national network)
  - life expectancy increased by 10 years; Causes of mortality: HIV 5.6% vs. CVD:27%

Invest in human resources, technical capacity for low income countries: the support of the Fogarty has been invaluable in Haiti

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The faces of Fogarty in Haiti

National HIV, TB, cholera GHESKIO models scaled-up nationwide through FIC training . GHESKIO credited with "slowing the AIDS epidemic in Haiti" and serving as "a model for how poor countries with few resources can combat AIDS, TB,and diarrhea" (NY Times)

Dosing MDRTB drugs in pediatric patients

Vanessa Rouzier, MD
Head of Pediatrics

HPV vaccination

Cynthia Riviere, MD,

OPINION

We have much to learn from research in Haiti

Long term ART complications

Samuel Pierre, MD

Oral cholera /Chikungunya vaccines

Karine Sévère, MD

Nitazonamide (NTZ) forMDRTB

Katey Walsh, MD

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Whither Fogarty?
Past 50 years and the Next 50 years

Sten H. Vermund
Yale School of Public Health

Yale SCHOOL OF PUBLIC HEALTH

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Fogarty International Clinical Research Scholars & Fellows+VIGH Fogarty training grants: 2005-2016

- Leveraged >$300 million in service/research grants, *e.g.*, PEPFAR & R01 grants
- Trainees published in *N Engl J Med, Lancet, JAMA, BMJ, PNAS, AIDS, JAIDS*

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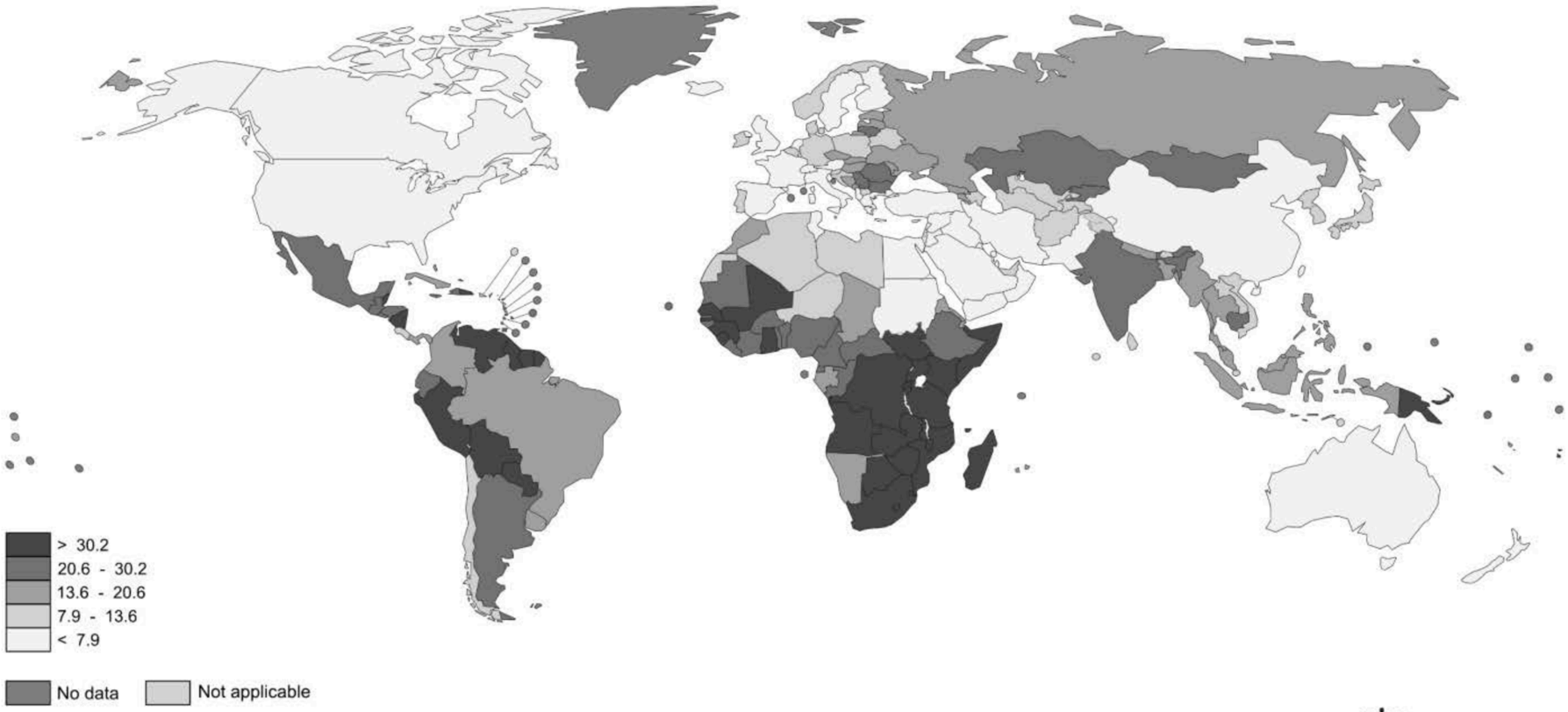
Impact case study: Cervical CA deaths, 2012



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

Data source: GLOBOCAN 2012
Map production: IARC
World Health Organization

World Health Organization

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Fogarty invests, the world responds

- In 2006, PEPFAR, Pink Ribbon Red Ribbon, and others supported CIDRZ, the Zambian Ministry of Health, and the University Teaching Hospital (UTH) to establish the Cervical Cancer Prevention Programme in Zambia
  - Fogarty trainee Dr Sharon Kapambwe seconded to MoH as Director of Women's Cancer Control in 2016
  - Sustainable national scale-up of cervical cancer and breast cancer screening and treatment throughout Zambia
  - Over 300,000 women screened for cervical cancer, 1/5 of the total in the last year alone
- Training in VIA-based screening and treatment for nurses/ doctors from >20 countries
- http://www.cidrz.org/cervical-cancer/ (publications of Parham, Mwanahamuntu, Sahasrabuddhe, Vermund, Kapambwe, et al

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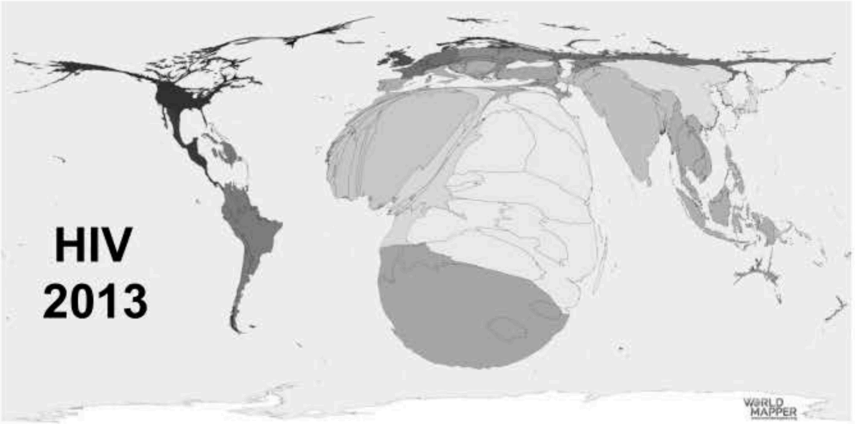
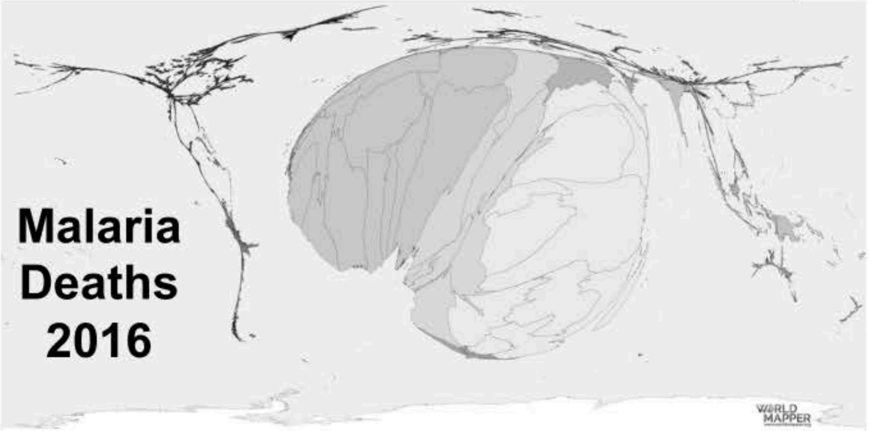
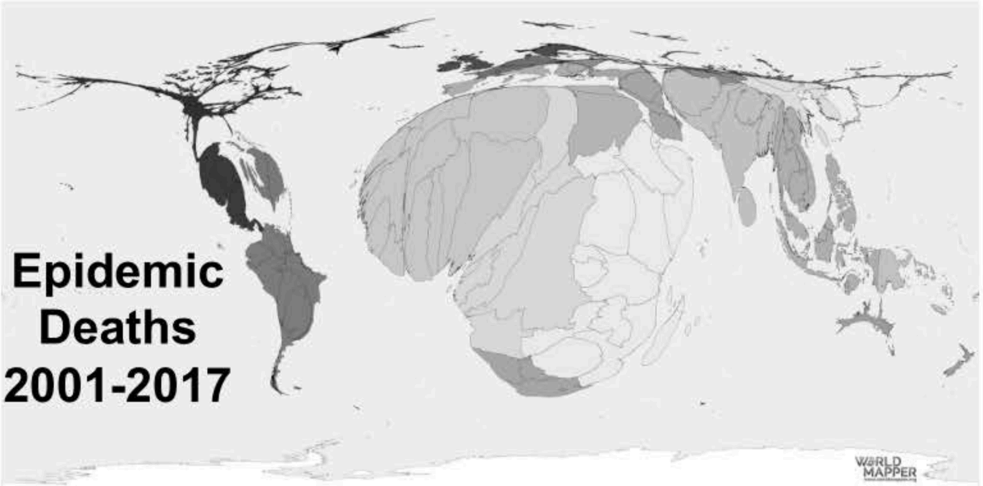
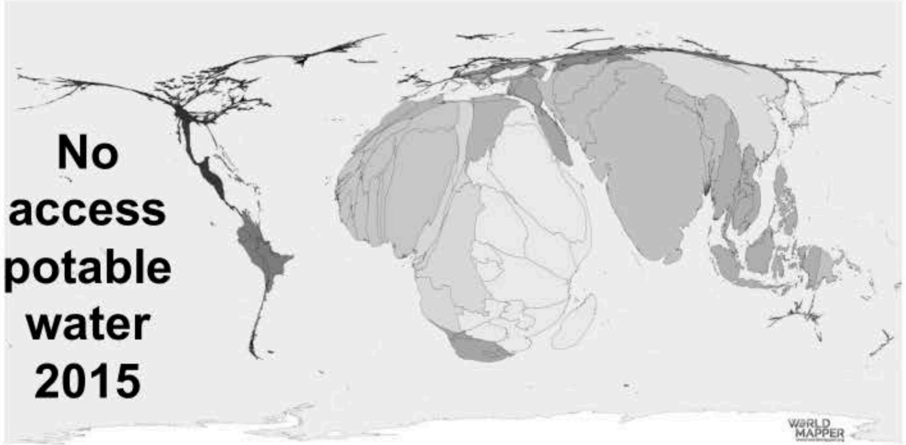
FIC past and future: Breman, Bridbord , Kupfer, Glass. Global health: the FIC/NIH. *Infect Dis Clin No Am* 2011









Cartograms from WorldMapper

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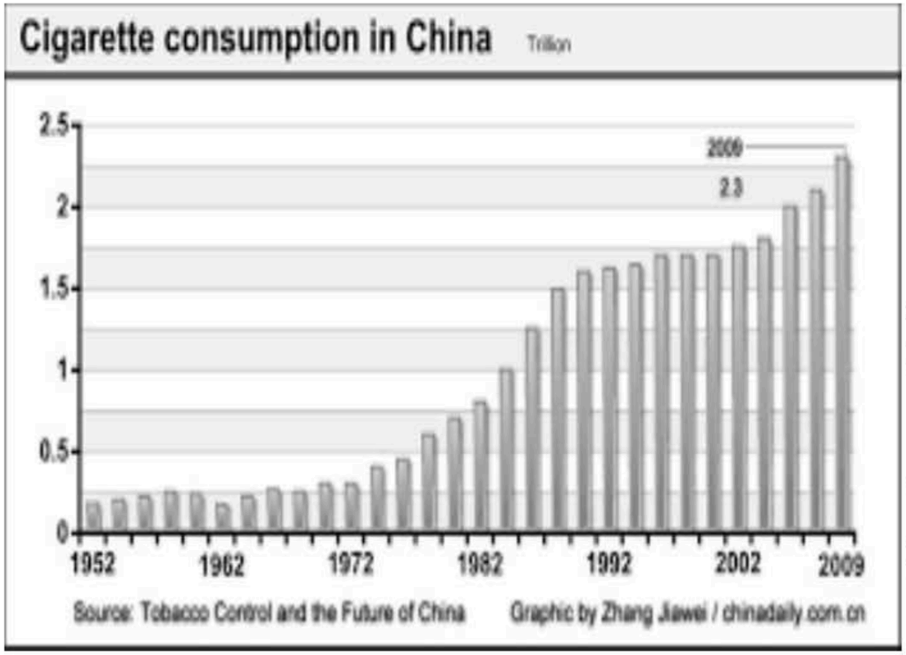
NCDs: Nutrition and Substance Use



New Kellogg's & General Mills Cereals introduced over the last six-months.

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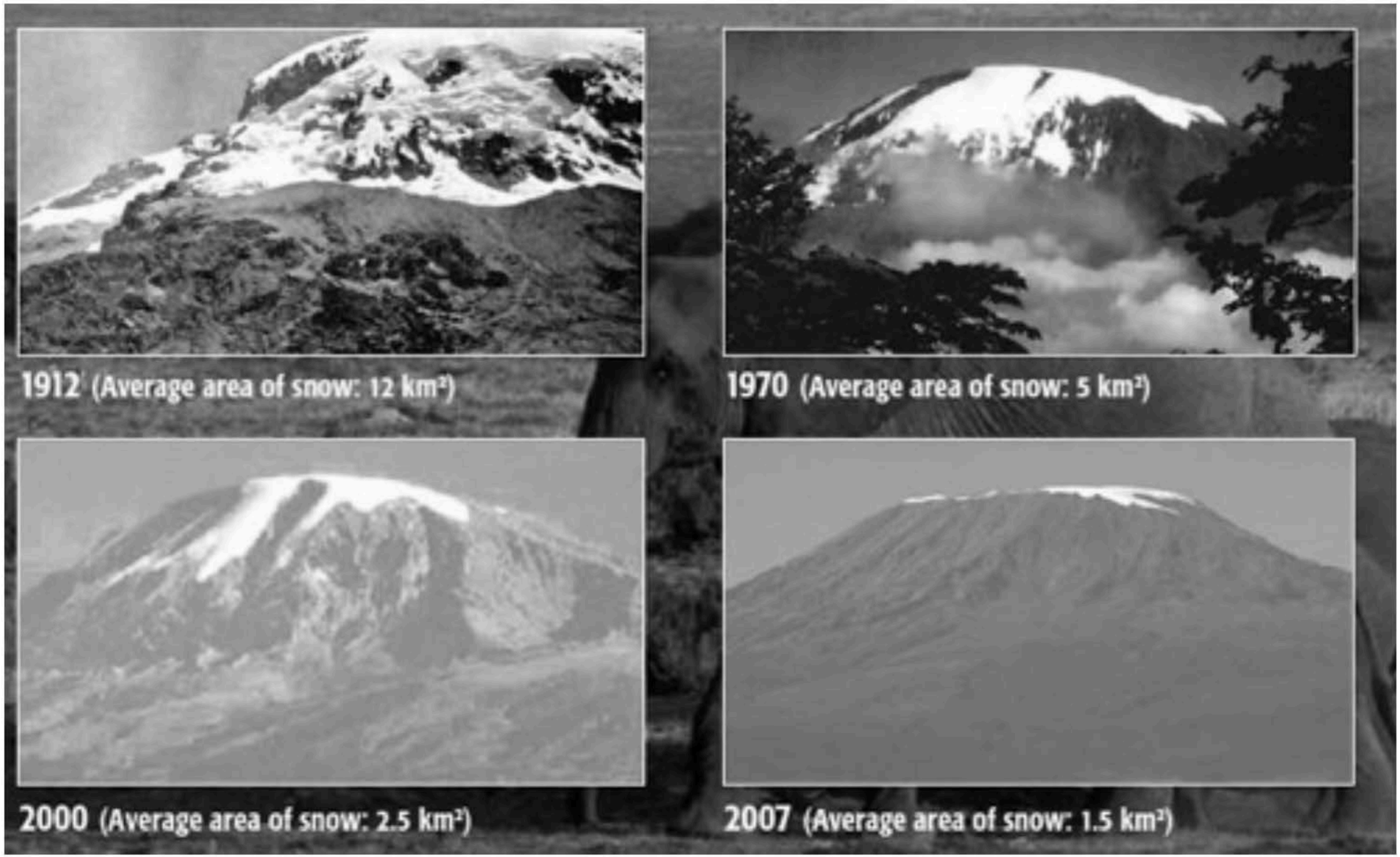

Debate: Deforestation vs. global warming.
Result in either case, malaria in the foothills

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Fogarty Future (Discovery and Implementation Science)

- Infectious disease prevention and control
- Non-communicable diseases globally, with attention to systemic forces that exacerbate poor nutrition, smoking, sedentary lifestyles
- Global warming with direct and indirect effects, with control of overpopulation by meeting the unmet need for contraception with the antecedent of low female literacy

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Yale SCHOOL OF PUBLIC HEALTH

(b)(6)

AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Mon, 30 Apr 2018 23:47:23 +0000
To: NIAID OD AM
Subject: FW: Favor of your reply requested || Keynote Speaker Invitation for the White House Fellows Annual Conference || October 25-26, 2018
Attachments: Invitation to Dr Fauci from the White House Fellows Foundation v3 (27 April 2018).pdf

Let us discuss. I know that I have Leadership Forum on October 25-26, but perhaps I could slip out for part of the day. We can figure out if I have any role in the forum and if so, when. Thanks.

From: Omenn, Gil (b)(6)
Sent: Monday, April 30, 2018 11:35 AM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: Favor of your reply requested || Keynote Speaker Invitation for the White House Fellows Annual Conference || October 25-26, 2018

Dear Tony,

Letter of invitation attached.
Best wishes,

Gilbert S. Omenn, MD, PhD
Harold T. Shapiro Distinguished University Professor
Director, Center for Computational Medicine & Bioinformatics
Professor of Computational Medicine & Bioinformatics, Internal Medicine, Human Genetics and Public Health
University of Michigan
Room 2017B Palmer Commons, 100 Washtenaw Avenue
Ann Arbor, MI 48109-2218
Ph: 734-763-7583 Cell: (b)(6) Fax: 734-615-6553
(b)(6)
www.ccmb.med.umich.edu/omenn
Assistant: Jane Wiesner (b)(6) / 734-615-4432)

****
Electronic Mail is not secure, may not be read every day, and should not be used for urgent or sensitive issues

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White House Fellows Foundation and Association
1750 Pennsylvania Ave NW, Suite 300, Washington, DC 20006
Main: 202-360-0294 Cell: (b)(6) Fax: 202-338-4384
david.moore@whff.org

April 27, 2018

Dr. Anthony S. Fauci, M.D.
National Institute of Health
National Institute of Allergy
NIAID Director
31 Center Drive
Bethesda MD 20894

Via Email

Keynote Speaker Invitation for the White House Fellows Annual Conference

Dear Tony:

On behalf of the White House Fellows Foundation and Association (WHFFA) Board of Directors, we are writing to invite you to be a keynote speaker at our Annual Leadership Conference, scheduled for October 25-26, 2018. The venue will be the U.S. Chamber of Commerce, at 1615 H Street, NW – directly across Lafayette Park from the White House. We expect more than 200 White House Fellows will attend. Your previous talk with the Fellows in 2013 was greatly appreciated.

The theme of our conference is "Character, Responsibility and Community: Creating Our Common Future". We would like a 20-minute keynote from you, Tony, followed by a panel discussion that may address pressing topics affecting domestic health, global health, and policy, such as HIV/AIDS, Ebola, other communicable diseases and outbreaks, and the Opioid epidemic. We would like to hear your thoughts on the leadership roles, responsibilities, actions and solutions needed to meet health challenges to create and sustain our common future.

We are inviting as panelists: Dr. Sanjay Gupta, Dr. Michelle Williams, and Rear Admiral Sylvia Trent-Adams, with Gil Omenn as moderator.

Please contact us by email (Stephanie: (b)(6) Gil: (b)(6) or by phone (Stephanie: (b)(6) Gil: (b)(6) to confirm your availability to participate in the conference. Ideally, we would like to schedule your remarks and the panel to follow for Friday morning, October 26, 2018 from 10:15am to 11:45am. That said, we will do our best to accommodate your schedule. We very much hope you will be able to join us.

Sincerely,

Stephanie L. Ferguson, PhD, RN, FAAN
Chair of the Conference Committee

Gil Omenn, MD, PhD
Leadership Conference Committee

AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Mon, 30 Apr 2018 23:23:51 +0000
To: Schwetz, Tara (NIH/NIAID) [E]
Cc: Tabak, Lawrence (NIH/OD) [E]
Subject: FW: Gates and POTUS

We shall discuss.

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Monday, April 30, 2018 10:06 AM
To: Collins, Francis (NIH/OD) [E] (b)(6)
Subject: RE: Gates and POTUS

Francis:
We should really talk by phone if possible. This is really disturbing. You and I have been talking about a universal influenza vaccine for a few years now https://www.ncbi.nlm.nih.gov/pubmed/21135852 . In June, 2017 we had our national and international Workshop on Universal Influenza vaccine that led to a brief summary of the workshop https://www.ncbi.nlm.nih.gov/pubmed/29045889 followed by our strategic plan and research agenda, which we also published https://www.ncbi.nlm.nih.gov/pubmed/29506129. Much of what Bill discussed came out of our plan and his visit to the VRC last year. In addition, we have been the lead in discussing the need for a universal influenza vaccine https://www.ncbi.nlm.nih.gov/pubmed/29185857 . In Bill's Shattuck Lecture on April 27, 2018, he mentioned "We are also supporting efforts by others including the National Institute of Allergy and Infectious Diseases, whose candidate vaccine is expected to advance to human safety trials in about a year". He is pledging a one time infusion of $12 million and we spend $104 million in our base per year with more to come. Happy to chat.
Best,
Tony

From: Collins, Francis (NIH/OD) [E]
Sent: Monday, April 30, 2018 9:27 AM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Tabak, Lawrence (NIH/OD) [E] (b)(6) Wolinetz, Carrie (NIH/OD) [E] (b)(6)
Subject: Gates and POTUS

Hi Tony,

See https://www.statnews.com/2018/04/30/bill-gates-vaccine-trump-meeting/

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Interesting report of a 40 minute meeting that Gates had with POTUS, where universal flu vaccine came up. POTUS called Gottlieb, who didn't have a lot to say. Gates doesn't seem to know there's a strategic plan. Should we be following up?

FC

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From: (b)(6)
Sent: Mon, 30 Apr 2018 19:12:01 -0400
To: Schwetz, Tara (NIH/NIAID) [E]
Subject: Re: Gates and POTUS

I am on my way

Sent from my iPad

On Apr 30, 2018, at 7:08 PM, Schwetz, Tara (NIH/NIAID) [E] (b)(6)

Ok, I'm heading back to campus because I forgot something. Happy to talk in person if you are still there.

Tara A. Schwetz, PhD
NIH/NIAID
(b)(6)
Sent from my iPhone

On Apr 30, 2018, at 6:57 PM, Fauci, Anthony (NIH/NIAID) [E] (b)(6)

(b)(6)

Sent from my iPad

Begin forwarded message:

From: "Tabak, Lawrence (NIH/OD) [E]" (b)(6)
Date: April 30, 2018 at 6:02:54 PM EDT
To: "Fauci, Anthony (NIH/NIAID) [E]" (b)(6)
Cc: "Collins, Francis (NIH/OD) [E]" (b)(6)
Subject: Gates and POTUS

Tony,
The Secretary took note of the lack of awareness about the universal flu vaccine effort at the WH. The Secretary will be meeting with POTUS on Wednesday and wants to be armed with the relevant bullet points of what we are doing (including the amount of money being spent). I could use the "1/2" pager by tomorrow morning please so the Secretary can make the "pitch" to the POTUS. Francis will be seeing the Secretary later in the afternoon tomorrow and he can reinforce.
Thanks,
Larry

--

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From: Vance, Meaghan (NIH/NIAID) [C]
Sent: Mon, 30 Apr 2018 22:48:21 +0000
To: Fauci, Anthony (NIH/NIAID) [E];Conrad, Patricia (NIH/NIAID) [E]
Subject: FW: question from Mother Jones

fyi

From: Kiera Butler [mailto:kbutler@motherjones.com]
Sent: Monday, April 30, 2018 5:59 PM
To: Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Subject: Re: question from Mother Jones

Thanks Meaghan—we actually just published our story. Next time!

Sent from my iPhone

On Apr 30, 2018, at 5:29 PM, Vance, Meaghan (NIH/NIAID) [C] (b)(6) wrote:

Dr. Fauci could call you at 7pm EST today. What's the best number to reach you on?

From: Kiera Butler [mailto:kbutler@motherjones.com]
Sent: Monday, April 30, 2018 1:50 PM
To: Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Subject: question from Mother Jones

Hi Meaghan,

Kiera at Mother Jones here—I'm writing a quick post on Trump's comments on universal flu vaccine with Bill Gates. Here's the STAT piece about it. Any chance Anthony Fauci might be willing to offer a quick comment today? He was really helpful when I was writing on universal flu vaccine research a few months ago!

Thanks!

Kiera

From: "Vance, Meaghan (NIH/NIAID) [C]" (b)(6)
Date: Thursday, March 22, 2018 at 3:44 PM
To: Kiera Butler (b)(6)
Subject: RE: interview with Dr. Fauci

Would you have time right now? Dr. Fauci has a few free minutes to talk....

From: Kiera Butler [mailto:kbutler@motherjones.com]
Sent: Thursday, March 22, 2018 3:43 PM

AMERICAN OVERSIGHT

To: Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Subject: Re: interview with Dr. Fauci

Hi Meaghan,

Thanks for getting back to me—yes, 2 PM tomorrow would work.

Best,

Kiera

From: "Vance, Meaghan (NIH/NIAID) [C]" (b)(6)
Date: Thursday, March 22, 2018 at 3:37 PM
To: Kiera Butler <kbutler@motherjones.com>
Subject: interview with Dr. Fauci

Kiera:

Dr. Fauci would be happy to speak with you about the new CDC director Robert Redfield and his background in HIV research. He has the following availability tomorrow:

8:30am, 9:30am or between 2-3pm

Will any of these times work?

Thank you,
Meaghan

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From: Billet, Courtney (NIH/NIAID) [E]
Sent: Mon, 30 Apr 2018 19:52:09 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Folkers, Greg (NIH/NIAID) [E];Conrad, Patricia (NIH/NIAID) [E];Stover, Kathy (NIH/NIAID) [E]
Subject: ASF: For your review. PR on launch of Phase 2 universal flu vaccine study
Attachments: biondvax release FOG cleared.docx, 14-0112_protocol_v3.0_29Jan18_eCTD.PDF, NSWB Clearance Sheet Biondvax flu trial.doc

Attached is our draft press release on the launch of a Phase 2 study of the BiondVax universal flu vaccine. There is a quote for you as follows:

"The 2017-2018 influenza season in the United States was among the worst of the last decade and serves as a reminder of the urgent need for a more effective and broadly protective influenza vaccine," said NIAID Director Anthony S. Fauci, M.D. "An effective universal influenza vaccine would lessen the public health burden of influenza, alleviate suffering and save lives. There are numerous paths of inquiry that the scientific community is pursuing, with each new study yielding more critical information and bringing us closer to our shared goal."

AMERICAN OVERSIGHT

DRAFT

FOR IMMEDIATE RELEASE
Friday, May 4, 2018
9 a.m. Eastern Time

Media Contact: Elizabeth Deatrick
(301) 402-1663
Email: elizabeth.deatrick@nih.gov

NIAID-Sponsored Trial of a Universal Influenza Vaccine Begins

A Phase 2 clinical trial of an investigational universal influenza vaccine intended to protect against multiple strains of the virus has begun in the United States. The study is sponsored by the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH), and is being conducted at four U.S. sites that are part of the NIAID-funded Vaccine and Treatment Evaluation Units (VTEUs). The trial is testing an experimental vaccine called M-001 for safety and its ability to produce potentially broad protective immune responses, both on its own and when followed by a standard licensed seasonal influenza vaccine.

Influenza viruses constantly mutate, resulting in the emergence of viruses that may not always match those targeted by seasonal and pre-pandemic influenza vaccines. Seasonal influenza vaccines are made anew each year to match the strains predicted to circulate in the upcoming season. To receive the best protection, people must be vaccinated annually against influenza. However, if a particular influenza strain changes in an unanticipated way, or a different strain from that included in the vaccine spreads widely, the seasonal influenza vaccine may not be sufficiently protective. Each year, seasonal influenza sickens millions in the United States and results in 140,000 to 710,000 hospitalizations and between 12,000 and 56,000 deaths, according to the U.S. Centers for Disease Control and Prevention (CDC).

Influenza pandemics occur when a novel influenza strain for which people have little to no protection begins to spread among humans and present a greater public health threat than seasonal influenza. For example, the 1918 influenza pandemic killed at least 50 million people worldwide. An ideal universal influenza vaccine would provide durable protection for all age groups against multiple influenza strains, including those that might cause a pandemic.

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"The 2017-2018 influenza season in the United States was among the worst of the last decade and serves as a reminder of the urgent need for a more effective and broadly protective influenza vaccine," said NIAID Director Anthony S. Fauci, M.D. "An effective universal influenza vaccine would lessen the public health burden of influenza, alleviate suffering and save lives. There are numerous paths of inquiry that the scientific community is pursuing, with each new study yielding more critical information and bringing us closer to our shared goal."

The new trial is being led by principal investigator Robert L. Atmar, M.D., of Baylor College of Medicine in Houston. The trial will test the M-001 vaccine candidate, developed and produced by BiondVax Pharmaceuticals based in Ness Ziona, Israel. The experimental M-001 vaccine contains antigenic peptide sequences shared among many different influenza viruses. Theoretically, it could protect against many current and emerging strains of influenza. Six previous clinical trials involving a total of 698 participants conducted by BiondVax in Israel and Europe indicated that the vaccine candidate was safe, well-tolerated and produced an immune response to a broad range of influenza strains.

The new study will enroll up to 120 healthy volunteers between the ages of 18 and 49 years. Participants will be assigned randomly to receive either two doses of the experimental vaccine or a placebo. They will be vaccinated twice, receiving one dose (1 mg; 0.4 milliliters) of M-001 or placebo via intramuscular injection on the first day and a second dose 22 days later. Approximately 172 days later, all participants will receive an approved seasonal influenza vaccine. During periodic additional clinic visits throughout the course of the study, blood will be drawn from study volunteers to evaluate their immune responses to both the experimental vaccine and to the seasonal vaccine. Each participant will be followed for approximately seven months.

The trial will take place at four NIAID-funded VTEUs. Patients will be enrolled at the Baylor College of Medicine; the University of Iowa in Iowa City; and Cincinnati Children's Hospital Medical Center. Laboratory support will be provided by Saint Louis University. For more information about the study, go to ClinicalTrials.gov, using the identifier NCT03058692.

NIAID conducts and supports research—at NIH, throughout the United States, and worldwide—to study the causes of infectious and immune-mediated diseases, and to develop better means of preventing, diagnosing and treating these illnesses. News releases, fact sheets and other NIAID-related materials are available on the NIAID website.

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A Phase II, Double-Blind, Multicenter, Randomized, Placebo-Controlled Trial to Assess the Safety, Reactogenicity and Immunogenicity of Two Doses of Multimeric-001 (M-001) Followed by Seasonal Trivalent Influenza Vaccine

DMID Protocol Number: 14-0112

DMID Funding Mechanism: Vaccine Treatment and Evaluation Units

Pharmaceutical Support Provided by:

BiondVax

IND Sponsor: Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health

Principal Investigator: Robert L. Atmar, M.D.

DMID Clinical Project Manager: Tena Knudsen, B.S.N.

DMID Medical Monitor: Soju Chang, M.D.

DMID Medical Officer: Trinka Coster, M.D.

DMID Scientific Lead: Chris Roberts, Ph.D.

Version Number: 3.0

29 January 2018

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HHS-NIAID-20-0593-A-001209

STATEMENT OF COMPLIANCE

The study will be carried out in accordance with Good Clinical Practices (GCP) as required by the following:

- United States Code of Federal Regulations (CFR) 45 CFR Part 46: Protection of Human Subjects
- Food and Drug Administration (FDA) Regulations, as applicable: 21 CFR Part 50 (Protection of Human Subjects), 21 CFR Part 54 (Financial Disclosure by Clinical Investigators), 21 CFR Part 56 (Institutional Review Boards), 21 CFR Part 11, and 21 CFR Part 312 (Investigational New Drug Application), 21 CFR 812 (Investigational Device Exemptions)
- International Council for Harmonisation: Good Clinical Practice (ICH E6); 62 Federal Register 25691 (1997); and future revisions
- Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, Report of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research
- National Institutes of Health (NIH) Office of Extramural Research, Research Involving Human Subjects, as applicable
- National Institute of Allergy and Infectious Diseases (NIAID) Clinical Terms of Award, as applicable
- Applicable Federal, State, and Local Regulations and Guidance

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SIGNATURE PAGE

The signature below provides the necessary assurance that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH E6 Good Clinical Practice (GCP) guidelines.

I agree to conduct the study in compliance with GCP and applicable regulatory requirements.

I agree to conduct the study in accordance with the current protocol and will not make changes to the protocol without obtaining the sponsor's approval and IRB/IEC approval, except when necessary to protect the safety, rights, or welfare of subjects.

Site Investigator Signature:

Signed: _____ Date: _____

Principal Investigator

Name:

Title:

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LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
AESIs	Adverse Events of Special Interest
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
CONSORT	Consolidated Standards of Reporting Trials
CFR	Code of Federal Regulations
CRF	Case Report Form
CRO	Contract Research Organization
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
FDA	Food and Drug Administration
FWA	Federalwide Assurance
GBS	Guillain-Barre Syndrome
GCP	Good Clinical Practice
HAI/HI	Hemagglutinin Inhibition
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IEC	Independent or Institutional Ethics Committee
IIV	Inactivated influenza vaccine
IIV3	Trivalent inactivated influenza vaccine
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
JAMA	Journal of the American Medical Association
M-001	Multimeric-001
MedDRA ®	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures

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N	Number (typically refers to subjects)
NCI	National Cancer Institute, NIH, DHHS
NDA	New Drug Application
NEJM	New England Journal of Medicine
Neut	Neutralization (refers to Neutralizing antibodies)
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS
OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
PBS	Phosphate Buffered Saline
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
PREP Act	Public Readiness and Emergency Preparedness Act
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
SDCC	Statistical and Data Coordinating Center
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
sub-I	Sub-Investigator
US	United States
WHO	World Health Organization

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PROTOCOL SUMMARY

Title:	A Phase II, Double-Blind, Multicenter, Randomized, Placebo-Controlled Trial to Assess the Safety, Reactogenicity and Immunogenicity of Two Doses of Multimeric-001 (M-001) Followed by Seasonal Trivalent Influenza Vaccine
Phase:	2
Population:	Up to 120 healthy males and non-pregnant females, aged 18 to 49 years (inclusive) will be enrolled.
Number of Sites:	Three sites: Baylor College of Medicine, the University of Iowa, and Cincinnati Children's Hospital Medical Center
Study Duration:	Approximately 24 months
Subject Participation Duration:	Approximately 7 months
Description of Agent or Intervention:	• M-001 manufactured by BiondVax • Licensed seasonal trivalent inactivated influenza vaccine (IIV3) • Normal saline placebo
Objectives:	Primary: <u>Safety</u> • To assess the safety as measured by vaccine related adverse events, reactogenicity, and laboratory adverse events of two doses of M-001 vaccine, each dose administered approximately 21 days apart <u>Immunogenicity</u> • To assess the T cell responses to M-001 component peptides following two doses of M-001

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Secondary:

Safety

- To assess all serious adverse events (SAEs) following receipt of each dose of M-001 vaccine or placebo, each dose separated by approximately 21 days, through the end of the study
- To assess all unsolicited non-serious adverse events (AEs) following receipt of each dose of M-001 or placebo, each dose separated by approximately 21 days, through 21 days after each dose of M-001 or placebo

Immunogenicity

- To assess the serum hemagglutination inhibition (HAI) and neutralizing (Neut) antibody responses to the 2018-2019 IIV3 vaccine viruses

Exploratory:

- To assess the IgG responses to the M-001 vaccine after receipt of placebo or two doses of M-001 vaccine and following receipt of IIV3
- To assess the T cell responses to M-001 component peptides following one dose of M-001 or placebo
- To assess T cell responses to M-001 component peptides following receipt of licensed IIV3
- To assess the T cell cross-reactive responses to current seasonal influenza virus antigens and pandemic influenza virus antigens following each vaccination (M-001 and IIV3)
- To assess the longevity of T cell responses to M-001 peptides, seasonal influenza virus antigens and pandemic influenza virus antigens

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Study Outcome Measures

Primary:

Safety

- Occurrence of vaccine-related SAEs from the time of the first study vaccination (M-001 or placebo) through approximately 6 months after the second M-001 study vaccination
- Occurrence of solicited injection site and systemic reactogenicity events on the day of each study vaccination through approximately 7 days after each M-001 vaccination
- Occurrence of clinical safety laboratory AEs from the time of each study vaccination of M-001 through approximately 7 days after each M-001 vaccination

Immunogenicity

- Geometric Mean Percentage of T cell subsets expressing perforin, CD107a, interferon gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), and interleukin 2 (IL2), alone or in combination, in CD4 and CD8 cells after stimulation with M-001 component peptides at baseline (Day 1) and 14 days after the second dose of M-001

Secondary:

Safety

- Occurrence of all SAEs, from the time of receiving the first M-001 or placebo study vaccination through the end of the study
- Occurrence of all unsolicited non-serious AEs from the time of first study vaccination through 21 days after each M-001 vaccination

Immunogenicity

- For HAI and Neuts, the percentage of subjects achieving seroconversion (defined as either a pre-vaccination titer <10 and a post-vaccination titer ≥40 or a pre-vaccination titer ≥10 and a minimum four-fold rise in post-

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vaccination antibody titer) to IIV3 vaccine viruses from Day 172 to Day 200

- For HAI and Neuts, the percentage of subjects with an antibody titer of 40 or greater and geometric mean titers (GMTs) vs. IIV3 vaccine viruses on Days 1, 43, 172 and 200

Exploratory:

- Percentage of subjects achieving seroconversion (defined as a minimum four-fold rise in post-vaccination ELISA antibody titer) and GMTs of ELISA antibody vs. M-001 from Day 1 to Days 22, 43, 78, 172 and 200

- Percentage of T cell subsets expressing perforin, CD107a, IFN-γ, TNF-α, and IL2, alone or in combination,in CD4 and CD8 cells after stimulation with M-001 peptides at baseline (Day 1) and approximately 14 days after one dose of M-001, 56 days following the second dose of M-001, and immediately prior to and 14 and 28 days after IIV3

- Percentage of T cell subsets expressing perforin, CD107a, IFN-γ, TNF-α, and IL2, alone or in combination, in CD4 and CD8 cells after stimulation with other influenza A antigens (including seasonal and pandemic influenza antigens) at baseline (Day 1), approximately 14 days after each study vaccination, 56 days following the second study vaccination and immediately prior to and approximately 28 days after IIV3

- Percentage of influenza-specific T cell memory subsets expressing discrete memory markers (CCR7, CD45RO, and CD57) at baseline (Day 1), approximately 56 days after the second dose of placebo or M-001 and immediately prior to and approximately 28 days after the IIV3 dose

Description of Study Design: This is a Phase II randomized, double-blind, placebo-controlled trial in 120 males and non-pregnant females, 18 to 49 years old, inclusive, who are in good health and meet all eligibility criteria. This clinical trial is designed to assess the safety, reactogenicity,

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and immunogenicity of two priming doses of M-001 followed by administration of IIV3.

Subjects will be assigned randomly to 1 of 2 treatment arms (60 subjects per treatment arm) to receive two doses of the M-001 vaccine or placebo (saline) followed by a single dose of IIV3 (2018-2019 formulation; see Table 1). Group A will receive two doses of M-001, each containing 1 mg of M-001, on Days 1 and 22, followed by a single dose of seasonal IIV3 on approximately Day 172 (window for this visit ranges from Day 142 to Day 202). Group B will receive saline placebo on Days 1 and 22, followed by a single dose of IIV3 on approximately Day 172.

Reactogenicity will be measured by the occurrence of solicited injection site and systemic reactions from the time of each study vaccination with M-001 (or placebo) through 7 days after the vaccination (inclusive of vaccination day). Unsolicited AEs will be collected from the time of each M-001 vaccination through approximately 21 days after each study vaccination. SAEs will be collected from the time of the first study vaccination through approximately 6 months after the second M-001 (or placebo) study vaccination. Clinical laboratory evaluations for safety will be performed on venous blood collected approximately 8 days after each M-001 (or placebo) vaccination (Days 9 and 30).

Immunogenicity testing will include performing HAI and Neut antibody assays on serum obtained immediately at baseline (Day 1) and on serum collected on Days 43, 172 and 200. ELISA antibody responses to the M-001 vaccine will be assessed on serum collected at baseline (Day 1) and on Days 22, 43, 172 and 200. Cell-mediated immune responses to influenza antigens, including epitopes represented in the M-001 vaccine, and seasonal and candidate pandemic influenza viruses, will be assessed at baseline (Day 1), 14 days after each vaccination (Days 15, 36, and 186), approximately 21 days (Day 43) and approximately 56 days (Day 78) following receipt of the second M-001 (or placebo) dose, and immediately prior to and approximately 28 days after the IIV3 dose (Days 172 and 200).

If visit 11 occurs before Study Day 200 (because Visit 9 occurred prior to Day 172), then an additional study visit (Visit 12, phone call) will occur on Day 200 to solicit SAE information.

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Estimated Time to Complete Enrollment: 2 to 3 months

Table 1: Treatment Arms

Treatment Arm	Dose 1 Day 1	Dose 2 Day 22	Dose 3 Day 172
A (n=60)	1 mg M-001	1 mg M-001	IIV3
B (n=60)	Placebo	Placebo	IIV3

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Schematic of Study Design:

Figure 1: Schematic of Study Design

Planned Enrollment: Total N=up to 120 subjects

Males and non-pregnant females, 18-49 years old, inclusive

↓

Consent

Assess Eligibility

↓

Randomize

↓

Treatment Arm	Dose 1 Day 1	Dose 2 Day 22	Dose 3 Day 172
A (n=60)	1 mg M-001	1 mg M-001	IIV3
B (n=60)	Placebo	Placebo	IIV3

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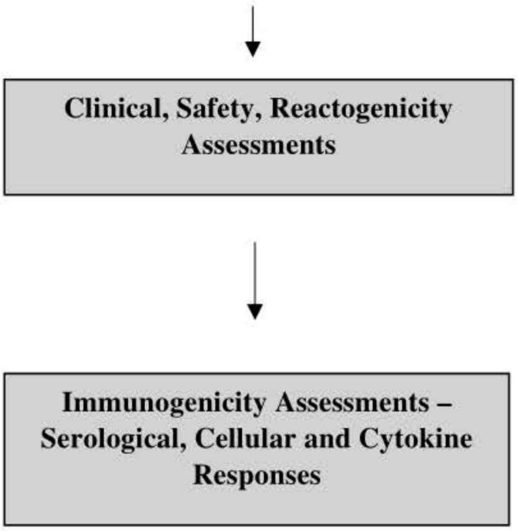

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1. KEY ROLES

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Statistical and Data Coordinating Center	Ashley Wegel The Emmes Corporation 401 N Washington Street, Suite 700 Rockville, MD 20850

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Study Agent Repository	Fisher BioServices c/o DMID Clinical Agents Repository Contract 20439 Seneca Meadows Parkway Germantown, MD 20876
Institutions:	Baylor College of Medicine, University of Iowa, Cincinnati Children's Hospital Medical Center
HAI and Neut Antibody Assays Laboratory	St Louis University 1100 S. Grand Boulevard St. Louis, MO 63104

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2. BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1. Background Information

2.1.1. Seasonal and pandemic influenza

Influenza is a common acute viral respiratory illness. Seasonal influenza occurs annually, and in the United States it causes an estimated 100 to 600 thousand hospitalization annually and up to 50,000 deaths a year (1-3). The continued emergence of novel influenza A viruses in humans—including subtypes H5N1, H3N2v, H7N7, H9N2, 2009 H1N1, and most recently H7N9, underscores the need for focused efforts to prepare for the next influenza pandemic (4-9). Four pandemics occurred during the last century. It was estimated that during the 1918 influenza A/H1N1 pandemic as many as 40 million deaths occurred worldwide (10). Excess mortality, high morbidity, and social disruption were all noted during the 1957 influenza A/H2N2 and the 1968 influenza A/H3N2 pandemics (11). In April 2009, a novel influenza virus (2009 A/H1N1) originated in pigs and spread to humans around the world becoming the first pandemic of this century. In each of these influenza pandemics, human populations lacked significant levels of pre-existing immunity to a highly transmissible form of the virus enabling it to spread rapidly. Thus, each emergence of a new subtype of influenza virus in the human population has the potential to result in a global public health emergency.

Use of influenza vaccines is the primary means for preventing influenza. Current licensed inactivated vaccines (IIVs) are good for preventing influenza, but are less effective than desirable.

Serum IgG antibody to the influenza virus HA, the major component of inactivated subunit and split virus vaccines, has a major role in protective immunity to influenza virus infection (12). Resistance to infection with seasonal influenza virus strains correlates directly with both serum hemagglutination inhibition (HAI) and neutralizing (Neut) antibody levels, and measurements of serum HAI and Neut antibodies are used routinely to assess the immunogenicity of both seasonal and pandemic IIVs. Cellular immunity also can have a role in preventing influenza-associated illness (13).

Other strategies to improve immune responses to potential pandemic strains are needed. One such approach includes the use of a vaccine that enhances cellular immunity against all influenza viruses (a universal vaccine) and that might also prime for serological responses to inactivated influenza antigens.

2.1.2. Multimeric-001 (M-001)

The use of epitope-based vaccines is another approach that may be used to prime for immune responses to influenza antigens (14). The M-001 vaccine from BiondVax consists of 3 repetitions of 9 conserved linear epitopes from the hemagglutinin, nucleoprotein and matrix 1

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protein that are prepared as a single recombinant protein. The epitopes in the vaccine are common to a large majority of influenza virus strains, and the epitopes are recognized by both the *humoral* and *cellular* arms of the immune system (14). Based upon these characteristics, the M-001 vaccine is hypothesized to provide priming immunity against both existing as well as future emergent seasonal and pandemic influenza virus strains.

The M-001 vaccine is produced as a recombinant protein in *E. coli*. In the current study, the vaccine is intended to be administered in a non-adjuvanted formulation at a 1 mg/dose level that has been found to be safe and immunogenic in previous clinical trials performed by BiondVax.

Preclinical studies using epitopes expressed in the vaccine in combination with adjuvant demonstrated both vaccine immunogenicity and protection from lethal challenge in a mouse model using highly pathogenic influenza A/H5N1 (15). The safety and efficacy results of M-001 in the preclinical studies led to its evaluation in people (16).

The initial study of M-001 in humans (BVX-002) was conducted in 63 healthy adults as a Phase 1 trial that evaluated the safety and immunogenicity of two doses of M-001 administered by the intramuscular (IM) route at 3 different dosage levels (125, 250 and 500 mcg) with or without Montanide ISA 51 VG adjuvant (17). No safety concerns were identified. Recipients of the adjuvanted 500 mcg dose of M-001 had 22-28% more frequent antibody-dependent, complement-mediated lysis of cells infected with influenza virus strains contained in seasonal vaccines compared to placebo recipients. This group also had significantly higher cellular (PBMC proliferation) responses after exposure to vaccine compared to unprimed groups.

The next study (BVX-003) performed in 60 older adults (55-75 years of age) evaluated the safety and ability of M-001 to improve humoral and cellular responses when used for priming prior to administration of a trivalent seasonal IIV [IIV3] (16). Participants (n=10 per group) received either Montanide ISA 51 VG-adjuvanted or non-adjuvanted M-001 or placebo 3 weeks prior to IIV3. A greater proportion of recipients of an adjuvanted 250 mcg M-001 dose (50-80%) had HAI seroresponses to the seasonal vaccine strains than did participants who only received IIV3 (20-30%). Following this study and based upon the lack of safety concerns, the 500 mcg dosage of M-001 was selected for further study.

BVX-004 evaluated the safety and immunogenicity of Montanide ISA 51 VG-adjuvanted M-001 when it was co-administered with IIV3 or used in a priming strategy in 200 healthy adults 18-49 years of age (16). Adjuvanted M-001, when administered twice before delivery of IIV3 and when administered in combination with 15% or 50% of standard dose IIV3 (either co-administered or in a prime-boost approach) was found to be safe and induced immune responses that exceeded those exhibited by subjects immunized with placebo or IIV3 alone.

The fourth study of M-001 (BVX-005) compared two priming doses of M-001 with one priming dose of alum-adjuvanted or non-adjuvanted M-001 administered 3 weeks prior to seasonal IIV3 in 120 elderly individuals (18). Seroconversion rates were significantly higher in the two-dose M-001 regimen for the seasonal A (H1N1) and B strains compared to placebo (IIV3 alone), and

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cellular immune responses (IFN-γ expressing CD4 and CD8 lymphocytes) were also significantly increased from pre-immunization levels after exposure to influenza antigens. No safety concerns were identified.

A fifth placebo-controlled study (BVX-006) examining two different dosage levels (0.5 mg and 1 mg) of M-001 as a three-dose prime for seasonal IIV3 immune responses has been conducted in 36 adults. No SAEs were observed and no safety concerns were identified (50). Elevated HAI responses were demonstrated in the experimental group receiving 1 mg M-001 before the IIV3 and hence, this dosage was selected for future trials.

2.1.3. Public Readiness and Emergency Preparedness Act

For this protocol the study product M-001 (manufactured by BiondVax Ltd.) is covered under the Public Readiness and Emergency Preparedness Act (PREP Act). Under the PREP Act, covered persons are immune from liability actions brought from the administration or use of a covered countermeasure that is the subject of a declaration. The PREP Act provides immunity for covered persons (such as manufacturers, distributers, program planners and other qualified persons who prescribe, administer or dispense the M-001 study vaccine) from tort liability, unless the injury was caused by willful misconduct.

The PREP Act also established the Countermeasures Injury Compensation Program (CICP) to provide compensation for serious injuries that occur as the result of the administration or use of certain countermeasures. Any requests for compensation must be filed within one year of administration or use of the countermeasure. Requests would go to the Health Resources and Services Administration (HRSA) Preparedness Countermeasures Injury Compensation Program (http://www.hrsa.gov/cicp/). Compensation may then be available for medical benefits, lost wages and death benefits to eligible individuals for specified injuries in accordance with regulations published by the Secretary of HRSA. Eligibility for compensation and the injuries for which compensation may be available are further defined by regulation.

An individual who suffers a serious physical injury or death from administration and use of the M-001 study vaccine may request benefits from CICP. A serious physical injury means an injury that is life threatening, results in, or requires medical or surgical intervention to prevent permanent impairment of a body function or permanent damage to body structure. The CICP is the payer of last resort. This means that it only covers expenses or provides benefits that other third-party payers, such as health insurance, the Department of Veterans Affairs, or Workers' Compensation programs don't have an obligation to pay.

If no funds have been appropriated to the compensation program, the Secretary of HRSA does not make a final determination on the individual's request within 240 days, or if the individual decides not to accept the compensation, the injured individual or his representative may pursue a tort claim in the United States District Court for the District of Columbia, but only if the claim involves willful misconduct, is pled with particularity required under the PREP Act, verified, and accompanied by an affidavit by a physician who did not treat the individual and certified medical

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records. Any award is reduced by any public or private insurance or worker's compensation available to the injured individual. Awards for non-economic damages, such as pain, suffering, physical impairment, mental anguish, and loss of consortium are also limited. If the individual accepts compensation, or if there is no willful misconduct, then the individual does not have a tort claim that can be filed in a United States Federal or a State court.

2.2. Rationale

Seasonal and pandemic influenza pose perpetual threats to human populations. The rapid and constant evolution of influenza viruses likewise poses challenges to the development of vaccines for the prevention and control of influenza. Recent vaccine development efforts have focused on the development of "universal" influenza vaccines; that is, vaccines which could offer protection against multiple influenza subtypes. BiondVax has developed a novel vaccine, M-001, that has been shown in preclinical and clinical trials to stimulate both the humoral and cellular arms of the immune system. When used as a primer ahead of a strain-specific boost, M-001 enhances immunity to strains contained within the boost and to drifted strains. The goals of the study are to determine the safety of a M-001 prime followed by an IIV3 boost vaccine strategy, and to assess the effects of M-001 immunization on cellular and humoral immunity to seasonal influenza virus strains.

2.3. Potential Risks and Benefits

2.3.1. Potential Risks

The potential risks of this study are those associated with having blood drawn, IM injection of the M-001 and IIV3 vaccines, possible reactions to the M-001 and trivalent seasonal influenza vaccines, and breach of confidentiality.

Drawing blood may cause transient discomfort and fainting. Fainting is usually transient and managed by having the subject lie down. Bruising at the blood draw site may occur, but can be prevented or lessened by applying pressure to the draw site for several minutes. IM injection also may cause transient discomfort and fainting. Drawing blood and IM injection may also cause infection. The use of sterile technique will make infection at the site where blood will be drawn or where the study vaccination is given extremely unlikely.

Occasionally, adult recipients of seasonal non-adjuvanted, licensed IIVs may develop influenza-like reactions such as fever, feverishness, fatigue, malaise, myalgia, arthralgia, headache, and/or nausea. Some subjects may develop reactions at the injection site, including pruritus, ecchymosis, erythema, induration /swelling, pain, and/or tenderness. With non-adjuvanted licensed, IIVs most of these reactions peak in intensity in the first 24 hours after vaccination and usually disappear without treatment within 1 or 2 days. Analgesics (e.g., acetaminophen, or ibuprofen or similar non-steroidal anti-inflammatory drugs (NSAIDs)) and rest may generally relieve or lessen these reactions. Bruising can sometimes occur due to the vaccination procedure.

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In addition, post-marketing surveillance indicates the following autoimmune, auto-inflammatory and immune-mediated diseases as potential risks for the seasonal IIVs: neuritis, convulsions, severe allergic reactions, syncope, encephalitis, thrombocytopenia, vasculitis, and Guillain-Barré syndrome. Reports of these reactions were rare; therefore, exact incidence rates cannot be precisely calculated.

Five previous trials in which approximately 309 participants received M-001 IM have been completed to date (16). AEs described following receipt of M-001 have been similar to those seen in placebo recipients but have included rhinitis/nasal congestion, malaise, myalgia, sore throat, injection site pain and erythema, cough, headache, diarrhea, neck pain, fever, back pain, arthralgia, nausea, vomiting, rash, flushing, dizziness and decreased blood pressure (16-18).

Acute and potentially life-threatening allergic reactions are also possible. Very rarely, occurring in about 1 in 4 million people given a licensed IIV, there can be a serious allergic reaction to a vaccine. These reactions can manifest as skin rash (hives), swelling around the mouth, throat or eyes, difficulty breathing, a fast pulse, or loss of blood pressure. If these reactions occur, they can usually be stopped by the administration of emergency medications by the study personnel. As with any vaccine or medication, there is a very small chance of a fatal reaction, although researchers do not expect this to occur.

During the swine influenza (H1N1) vaccine campaign of 1976, some recipients developed a paralytic illness called Guillain-Barré syndrome (GBS). GBS is an acute inflammatory neuropathy characterized by weakness, hyporeflexia or areflexia, and elevated protein concentrations in cerebrospinal fluid. The rate of GBS was significantly increased in individuals receiving the 1976 swine influenza (H1N1) vaccine at about 1 per 100,000 vaccine recipients. This syndrome has not been seen consistently with other influenza vaccines. Most persons who develop GBS recover completely, although the recovery period may be as little as a few weeks or as long as a few years. About 30% of those with GBS still have residual weakness after 3 years and about 3% may suffer a relapse of muscle weakness and tingling sensations many years after the initial attack. Intensive surveillance of GBS after administration of IIVs since 1976 has shown a slight increase in risk over background cases (more than one additional case of GBS per million persons) following vaccination, typically with onset within 6 weeks after vaccination (19). Interestingly, although vaccination rates have increased in the last 10 years the numbers of reported cases of vaccine-associated GBS have declined (20). A recent study in Canada showed that the 2009 H1N1 vaccine was associated with a small but significant risk of GBS in persons 50 years and older (21). An active, population-based surveillance study conducted during the 2009-2010 influenza season found less than 1 excess GBS case per million doses of 2009 H1N1 vaccine administered – a rate similar to that associated with some previously administered annual influenza vaccines (22-25). Another study using the Medicare system showed an elevated risk of GBS with 2009 monovalent H1N1 vaccination (incidence rate ratio = 2.41, 95% confidence interval: 1.14, 5.11; attributable risk = 2.84 per million doses administered, 95% confidence interval: 0.21, 5.48) (26). An international collaboration study also supported a conclusion of an association between 2009 H1N1 vaccination and GBS (27).

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It is unknown if the M-001 vaccine poses any risks to an unborn child. Female subjects of childbearing potential who are not surgically sterile via tubal sterilization, bilateral oophorectomy, hysterectomy, or successful Essure® placement with documented radiological confirmation at least 90 days after the procedure, or who are not postmenopausal for ≥ 1 year, must agree to practice highly effective contraception that may include, but is not limited to, abstinence from intercourse with a male partner, monogamous relationship with a vasectomized partner, male condoms with the use of applied spermicide, intrauterine devices, and licensed hormonal methods, for a minimum of 30 days prior to study product through 2 months (defined as 60 days) after the last dose of M-001 or placebo. A highly effective method of contraception is defined as one which results in a low failure rate (i.e., less than 1% per year) when used consistently and correctly. In addition to contraceptive use, all female subjects of childbearing potential will be required to have a negative serum or urine pregnancy test within 24 hours prior to receiving each dose of study vaccine. If a female subject becomes pregnant while participating in this study, we will ask her permission to follow-up with her about her health and the health of her baby through pregnancy outcome.

Subjects will be asked to provide personal health information (PHI). All attempts will be made to keep this PHI confidential within the limits of the law. However, there is a chance that unauthorized persons will see the subjects' PHI. All records will be kept in a locked file cabinet or maintained in a locked room at the participating VTEU sites. Electronic files will be password protected. Only people who are involved in the conduct, oversight, monitoring, or auditing of this study will be allowed access to the PHI that is collected. Any publications from this study will not use information that will identify subjects by name. Organizations that may inspect and/or copy research records maintained at the participating VTEU sites for quality assurance and data analysis include groups such as NIAID and FDA.

There may be other unknown risks, discomforts, or side effects.

2.3.2. Known Potential Benefits

There are no known benefits attributable to the receipt of the M-001 vaccine, but there is the prospect of benefit. It is possible that vaccination with M-001 will prime the participant for improved responses to future seasonal or pandemic influenza vaccines. Vaccination with IIV3 likely will lead to protection against seasonal influenza viruses.

There may be pandemic preparedness benefits to society in the future if the M-001 is shown to have a significant priming effect that leads to improved cellular immune responses to influenza and proves to be sufficiently safe so that it can be employed if a need for widespread vaccination against pandemic influenza virus strains occurs.

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3. OBJECTIVES

3.1. Study Objectives

3.1.1. Primary Objectives

Safety

- To assess the safety as measured by vaccine related adverse events, reactogenicity, and laboratory adverse events of two doses of M-001 vaccine, each dose administered approximately 21 days apart

Immunogenicity

- To assess the T cell responses to M-001 component peptides following two doses of M-001

3.1.2. Secondary Objectives

Safety

- To assess all serious adverse events (SAEs) following receipt of each dose of M-001 vaccine or placebo, each dose separated by approximately 21 days, through the end of the study
- To assess all unsolicited non-serious AEs following receipt of each dose of M-001 or placebo, each dose separated by approximately 21 days, through 21 days after each dose of M-001 or placebo

Immunogenicity

- To assess the serum HAI and Neut antibody responses to the 2018-2019 IIV3 vaccine viruses

3.1.3. Exploratory Objectives

- To assess the IgG responses to the M-001 vaccine after receipt of placebo or two doses of M-001 vaccine and following receipt of licensed IIV3
- To assess the T cell responses to M-001 component peptides following one dose of M-001 or placebo
- To assess T cell responses to M-001 component peptides following receipt of licensed IIV3

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- To assess the T cell cross-reactive responses to current seasonal influenza virus antigens and pandemic influenza virus antigens following each vaccination (M-001 and IIV3)

- To assess the longevity of T cell responses to M-001 peptides, seasonal influenza virus antigens and pandemic influenza virus antigens

3.2. Study Outcome Measures

3.2.1. Primary Outcome Measures

Safety

- Occurrence of vaccine-related SAEs from the time of the first study vaccination (M-001 or placebo) through approximately 6 months after the second M-001 study vaccination

- Occurrence of solicited injection site and systemic reactogenicity events on the day of each study vaccination through approximately 7 days after each M-001 vaccination

- Occurrence of clinical safety laboratory adverse events from the time of each study vaccination of M-001 through approximately 7 days after each M-001 vaccination

Immunogenicity

- Geometric Mean Percentage of T cell subsets expressing perforin, CD107a, IFN-γ, TNF-α, and IL2, alone or in combination, in CD4 and CD8 cells after stimulation with M-001 component peptides at baseline (Day 1) and 14 days after the second dose of M-001

3.2.2. Secondary Outcome Measures

Safety

- Occurrence of all SAEs, from the time of receiving the first M-001 or placebo study vaccination through the end of the study

- Occurrence of all unsolicited non-serious AEs from the time of first study vaccination through 21 days after each M-001 vaccination

Immunogenicity

- For HAI and Neuts, the percentage of subjects achieving seroconversion (defined as either a pre-vaccination titer <10 and a post-vaccination titer ≥40 or a pre-

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vaccination titer ≥10 and a minimum four-fold rise in post-vaccination antibody titer) to IIV3 vaccine viruses from Day 172 to Day 200

- For HAI and Neuts, the percentage of subjects with an antibody titer of 40 or greater and GMTs vs. IIV3 vaccine viruses on Days 1, 43, 172 and 200

3.2.3. Exploratory Outcome Measures

- Percentage of subjects achieving seroconversion (defined as a minimum four-fold rise in post-vaccination ELISA antibody titer) and GMTs of ELISA antibody vs. M-001 from Day 1 to Days 22, 43, 78, 172 and 200

- Geometric Mean Percentage of T cell subsets expressing perforin, CD107a, IFN-γ, TNF-α, and IL2, alone or in combination,in CD4 and CD8 cells after stimulation with M-001 peptides at baseline (Day 1) and approximately 14 days after one dose of M-001, 56 days following the second dose of M-001, and immediately prior to and 14 and 28 days after IIV3

- Percentage of T cell subsets expressing perforin, CD107a, IFN-γ, TNF-α, and IL2, alone or in combination, in CD4 and CD8 cells after stimulation with other influenza A antigens (including seasonal and pandemic influenza antigens) at baseline (Day 1), approximately 14 days after each study vaccination, 56 days following the second study vaccination and immediately prior to and approximately 28 days after IIV3

- Percentage of influenza-specific T cell memory subsets expressing discrete memory markers (CCR7, CD45RO, and CD57) at baseline (Day 1), approximately 56 days after the second dose of placebo or M-001 and immediately prior to and approximately 28 days after the IIV3 dose

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4. STUDY DESIGN

This is a Phase II randomized, double-blind, placebo-controlled trial in 120 males and non-pregnant females, 18 to 49 years old, inclusive, who are in good health and meet all eligibility criteria. This clinical trial is designed to assess the safety, reactogenicity, and immunogenicity of two priming doses of M-001 followed by administration of IIV3.

Subjects will be assigned randomly to 1 of 2 treatment arms (60 subjects per treatment arm) to receive two doses of the M-001 vaccine or placebo (saline) followed by a single dose of IIV3 (see Table 1). Group A will receive two doses of M-001, each containing 1 mg of M-001, on Days 1 and 22 followed by a single dose of IIV3 on approximately Day 172 (window for this visit ranges from Day 142 to Day 202). Group B will receive saline placebo on both Days 1 and 22, followed by a single dose of IIV3 approximately on Day 172.

Reactogenicity will be measured by the occurrence of solicited injection site and systemic reactions from the time of each study vaccination with M-001 (or placebo) through 7 days after the vaccination (inclusive of vaccination day). Unsolicited AEs will be collected from the time of each M-001 (or placebo) vaccination through approximately 21 days after each study vaccination. SAEs will be collected from the time of the first study vaccination through approximately 6 months after the second M-001 (or placebo) study vaccination. Clinical laboratory evaluations for safety will be performed on venous blood collected approximately 8 days after each M-001 (or placebo) vaccination (Days 9 and 30).

Immunogenicity testing will include performing HAI and Neut antibody assays on serum obtained immediately at baseline (Day 1) and on serum collected on Days 43, 172 and 200. ELISA antibody responses to the M-001 vaccine will be assessed on serum collected at baseline (Day 1) and on Days 22, 43, 172 and 200. Cell-mediated immune responses to influenza antigens, including epitopes represented in the M-001 vaccine, and seasonal and candidate pandemic influenza viruses, will be assessed at baseline (Day 1), 14 days after each vaccination (Days 15, 36, and 186), approximately 21 days (Day 43) and approximately 56 days (Day 78) following receipt of the second M-001 (or placebo) dose, and immediately prior to and approximately 28 days after the IIV3 dose (Days 172 and 200).

The duration of this trial for each subject will be approximately 7 months. If visit 11 occurs before Study Day 200 (because Visit 9 occurred prior to Day 172 and moved visits 10 and 11 to earlier times after study start), then an additional study visit (Visit 12, phone call) will occur on Day 200 to solicit SAE information.

For additional details on study procedures and evaluations and study schedule by study visits/days, see Sections 7 and 8 and Appendix A: Schedule of Study Procedures and Evaluations.

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5. STUDY ENROLLMENT AND WITHDRAWAL

5.1. Subject Inclusion Criteria

Subjects eligible to participate in this study must meet all of the following inclusion criteria:

1. Provide written informed consent prior to initiation of any study procedures.
2. Are able to understand and comply with planned study procedures and be available for all study visits.
3. Are males or non-pregnant females, 18 to 49 years old, inclusive.
4. Are in good health*.

   As determined by medical history and targeted physical examination to evaluate acute or currently ongoing chronic medical diagnoses or conditions, defined as those that have been present for at least 90 days, which would affect the assessment of the safety of subjects or the immunogenicity of study vaccinations. Chronic medical diagnoses or conditions should be stable for the last 60 days. This includes no change in chronic prescription medication, dose, or frequency as a result of deterioration of the chronic medical diagnosis or condition in the 60 days prior to enrollment. Any prescription change that is due to change of health care provider, insurance company, etc., or that is done for financial reasons, as long as in the same class of medication, will not be considered a deviation of this inclusion criterion. Any change in prescription medication due to* ***improvement* *of a disease outcome, as determined by the site principal investigator or appropriate sub-investigator, will not be considered a deviation of this inclusion criterion. Subjects may be on chronic or as needed (prn) medications if, in the opinion of the site principal investigator or appropriate sub-investigator, they pose no additional risk to subject safety or assessment of reactogenicity and immunogenicity and do not indicate a worsening of medical diagnosis or condition. Similarly, medication changes subsequent to enrollment and study vaccination are acceptable provided there was no deterioration in the subject's chronic medical condition that necessitated a medication change, and there is no additional risk to the subject or interference with the evaluation of responses to study vaccination. Note: Topical, nasal, and inhaled medications (with the exception of inhaled corticosteroids as outlined in the Subject Exclusion Criteria (see Section 5.1.2), herbals, vitamins, and supplements are permitted.*

5. Oral temperature is less than 100.0°F.
6. Pulse is 50 to 100 bpm, inclusive.
7. Systolic blood pressure is 85 to 150 mm Hg, inclusive.
8. Diastolic blood pressure is 55 to 95 mmHg, inclusive.
9. Women of childbearing potential‡ must use an acceptable method of contraception§ from 30 days prior to vaccination until 60 days after the second of dose of M-001 or placebo.
10. Women of childbearing potential‡ must use an acceptable method of contraception§ from 30 days prior to receipt of IIV vaccination, and must plan to use until 28 days after the IIV.

    ‡Not sterilized via tubal ligation, bilateral oophorectomy, hysterectomy, or successful Essure® placement (permanent, non-surgical, non-hormonal sterilization with history of documented radiological confirmation test achieved or with use of another approved birth control method if confirmation test not confirmed) and still menstruating or < 1 year of the last menses if menopausal).

    §Includes, but is not limited to, non-male sexual relationships, abstinence from sexual intercourse with a male partner, monogamous relationship with a vasectomized partner, male condoms with the use of applied spermicide, intrauterine

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devices, NuvaRing®, and licensed hormonal methods such as implants, injectables, contraceptive patches or oral contraceptives ("the pill"). Method of contraception will be captured on the appropriate data collection form.

11. Female subjects of childbearing potential must have a negative urine or serum pregnancy test within 24 hours prior to study vaccination.

5.2. Subject Exclusion Criteria

Subjects eligible to participate in this study must not meet any of the following exclusion criteria:

1. Have an acute illness[1], as determined by the site PI or appropriate sub-investigator, within 72 hours prior to study vaccination.

   [1]An acute illness which is nearly resolved with only minor residual symptoms remaining is allowable if, in the opinion of the site PI or appropriate sub-investigator, the residual symptoms will not interfere with the ability to assess safety parameters as required by the protocol.

2. Have any medical disease or condition that, in the opinion of the site PI or appropriate sub-investigator, is a contraindication to study participation[2].

   [2]Including acute or chronic medical disease or condition, defined as persisting for at least 90 days, that would place the subject at an unacceptable risk of injury, render the subject unable to meet the requirements of the protocol, or may interfere with the evaluation of responses or the subject's successful completion of this study.

3. Have immunosuppression as a result of an underlying illness or treatment, or use of anticancer chemotherapy or radiation therapy (cytotoxic) within 3 years prior to study vaccination.
4. Have known active neoplastic disease or a history of any hematologic malignancy. Non-melanoma skin cancers that are not active are permitted.
5. Have known HIV, hepatitis B, or hepatitis C infection.
6. Have known hypersensitivity or allergy to eggs, egg or chicken protein, or other components of the study vaccine.
7. Have a history of severe reactions following previous immunization with licensed or unlicensed influenza vaccines.
8. Have a history of Guillain-Barré Syndrome.
9. Have a history of alcohol or drug abuse within 5 years prior to study vaccination.
10. Have any diagnosis, current or past, of schizophrenia, bipolar disease, or other psychiatric diagnosis that may interfere with subject compliance or safety evaluations.
11. Have been hospitalized for psychiatric illness, history of suicide attempt, or confinement for danger to self or others within 10 years prior to study vaccination.
12. Have taken oral or parenteral (including intraarticular) corticosteroids of any dose within 30 days prior to study vaccination.

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13. Have taken high-dose[3] dose inhaled corticosteroids within 30 days prior to study vaccination[4].

    [3]*High-dose defined as per age as using inhaled high dose per reference chart https://www.nhlbi.nih.gov/health-pro/guidelines/current/asthma-guidelines/quick-reference-html#estimated-comparative-daily-doses.*

    [4]*Topical and nasal steroids are permissible.*

14. Received any licensed live vaccine or plan to receive a licensed live vaccine within 30 days prior or 21 days after each M-001 study vaccination.

15. Received a licensed inactivated vaccine within 14 days prior to or 21 days after each M-001 study vaccination.

16. Plans to or received the current 2018-2019 influenza vaccine (inactivated or live prior to orduring the study (the 2018-2019 influenza vaccine will be given during the trial.)

17. Received immunoglobulin or other blood products (with exception of Rho D immunoglobulin) within 90 days prior to study vaccination.

18. Received an experimental agent[5] within 30 days prior to the first study vaccination, or expects to receive an experimental agent[6] during the 15-month trial-reporting period.
    [5]*Including vaccine, drug, biologic, device, blood product, or medication.*

    [6]*Other than from participation in this study.*

19. Are participating or plan to participate in another clinical trial with an interventional agent[7] that will be received during the 7-month trial-reporting period.

    [7]*Including agent (licensed or unlicensed vaccine, drug, biologic, device, blood product, or medication) during the 9-month study period.*

20. Plan to travel outside the U.S. (continental U.S., Hawaii and Alaska) in the time between the first study vaccination and 21 days after the last study vaccination.

21. Female subjects who are breastfeeding or plan to breastfeed at any given time from the first study vaccination until 30 days after the last study vaccination.

22. Blood donation or planned blood donation within 30 days prior to the study vaccination through 30 days after the last blood drawn for this study.

23. Have signs or symptoms that could confound or confuse assessment of study vaccine reactogenicity[9].

    [9]*The study vaccination should be postponed/deferred until signs or symptoms have resolved and if within the acceptable protocol-specified window for that visit.*

24. Have a history of convulsions or encephalomyelitis within 90 days prior to study vaccination.

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5.3. Eligibility Criteria for Doses 2- 3

Subjects must meet all inclusion and none of the exclusion criteria as outlined in sections 5.1 and 5.2 for all subsequent vaccination visits. Subjects must not have received the 2018-2019 influenza vaccine prior to Dose #3. In addition, the presence of any unresolved or continuing solicited or unsolicited Grade 2 or 3 AE (Doses #2 to #3) or the presence of any Grade 2 or 3 clinical laboratory value (prior to Doses #2 or #3 administered on Days 22 and 172, respectively), will be exclusions to subsequent doses of vaccine. An unresolved or continuing Grade 1 AE is permissible unless, in the opinion of the site PI or sub-investigator (sub-I), it would render study vaccination unsafe or interfere with the evaluation of responses.

5.4. Treatment Assignment Procedures

5.4.1. Randomization Procedures

Per International Conference on Harmonisation (ICH) guideline E6: Good Clinical Practice (GCP), screening records will be kept at each participating VTEU site to document the reason why an individual was screened, but failed trial entry criteria. The reasons why individuals failed screening will be recorded in the Statistical and Data Coordinating Center's (SDCC) AdvantageEDC^{SM} (Electronic Data Capture System).

Once consented and upon entry of demographic data and confirmation of eligibility for this trial, the subject will be enrolled. One hundred twenty subjects will be randomly assigned to one of 2 treatment arms, as shown in Table 1. Randomization will be stratified based upon prior receipt of the 2017-2018 influenza vaccine. The list of randomized treatment assignments will be prepared by statisticians at the SDCC and included in the enrollment module for this trial. AdvantageEDC will assign each volunteer a treatment code from the list after demographic and eligibility data have been entered into the system. A designated individual at each site will be provided with a treatment key, which links the treatment code to the actual treatment assignment, which will be kept in a secure place.

Instructions for use of the enrollment module are included in the AdvantageEDC User's Guide. Manual back-up randomization procedures are provided in the MOP for use in the event that the site temporarily loses access to the Internet or the online enrollment system is unavailable.

The randomization scheme for this study is presented in Table 1.

5.4.2. Masking Procedures

This is a double-blind study.

Subjects, investigators, and study personnel performing any study-related assessments following study injection will be blinded to treatment assignment to the two M-001 dosing regimen or placebo. Laboratory personnel performing immunology assays will be blinded to treatment assignment.

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The randomization scheme will be generated by the SDCC and provided to unblinded study personnel (i.e., pharmacists performing study product preparations and unblinded study product administrators).

The unblinded study product administrator is a study personnel licensed, registered, or certified to administer medications/vaccines, but will not be involved in study-related assessments or have subject contact for data collection following study injection.

The Data and Safety Monitoring Board (DSMB) may receive data in aggregate and by treatment arm, or may be unblinded to individual subject treatment assignments, as needed, to adequately assess safety issues.

Refer to the MOP for unblinding procedures.

5.4.3. Reasons for Withdrawal

Subjects may voluntarily withdraw their consent for study participation at any time and for any reason, without penalty.

A subject may withdraw or be withdrawn from the study for any of the following reasons:

- Medical disease or condition, or any new clinical findings for which continued participation, in the opinion of the site PI or appropriate sub-I, would compromise the safety of the subject, or would interfere with the subject's successful completion of the study, or would interfere with the evaluation of responses.
- Subject no longer meets eligibility criteria.
- As deemed necessary by the site PI or appropriate sub-investigator for noncompliance or other reasons.
- Subject withdrawal of consent.
- Subject lost to follow-up.
- Termination of the study.
- New information becomes available that makes further participation unsafe.

Subsequent study vaccinations will not be administered to a subject if any of the following criteria are met:

- Medical condition for which continued participation, in the opinion of the site PI or appropriate sub-investigator, would pose a risk to the subject or would be likely to confound interpretation of the results.
- Presence of signs or symptoms that could confound or confuse assessment of study vaccine reactogenicity. For subjects with injection site or systemic signs or symptoms, or

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with an acute illness, including an oral temperature greater than or equal to 100.0°F, the subsequent study vaccinations should be postponed/deferred until signs, symptoms, or acute illness have resolved and if within the acceptable protocol-specified window for that visit. If outside this window, the DMID Medical Officer must first approve the subsequent study vaccination and the documentation of approval should be filed in the subject's chart.

- Any unresolved or continuing solicited or unsolicited Grade 2 or 3 AE. An unresolved or continuing Grade 1 AE is permissible unless, in the opinion of the site PI or sub-I, it would render study vaccination unsafe or interfere with the evaluation of responses.
- Grade 2 or 3 clinical safety laboratory value (according to the toxicity table, Section 9.2.3) that does not decrease to Grade 1 or less prior to any subsequent study vaccination. Any clinical safety laboratory parameter may be re-evaluated for eligibility only once at the central (clinical) laboratory prior to any subsequent study vaccination. If the clinical safety laboratory value decreases to Grade 1 or less, the subject may receive the subsequent study vaccination. Subsequent study vaccinations should be scheduled to occur within the acceptable protocol-specified window for that visit.
- Grade 3 AE in the 8 days following a study vaccination that has no alternative etiology.
- Subject no longer meets eligibility criteria.
- As deemed necessary by the site PI or appropriate sub-I for noncompliance or other reasons.
- Subject refusal of further study vaccination.
- Subject withdrawal of consent.
- Subject lost to follow-up.
- Termination of the study.
- New information becomes available that makes further participation unsafe.

5.4.4. Handling of Withdrawals

The primary reason for withdrawal from the study will be recorded on the Study Status data collection form. Subjects will be encouraged to complete the Early Termination Visit. The Early Termination Visit procedures are listed in Section 7.4. Although subjects are free to withdraw at any time or may be withdrawn by the site PI or appropriate sub-I at any time, subjects who receive at least one dose of study vaccine will be encouraged to remain in the study for follow-up safety assessments and collection of venous blood samples for immunogenicity testing. Every

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attempt will be made to follow to resolution all AEs, including solicited injection site and systemic reactions, and/or SAEs that are ongoing at the time of early withdrawal.

In the case of subjects who fail to appear for a follow-up safety assessment, extensive effort (i.e., three documented contact attempts via phone calls, e-mails, etc., made on separate occasions and followed by a certified letter) will be made to locate or recall them, or at least to determine their health status. These efforts will be documented in the subjects' records. See the protocol-specific Manual of Procedures (MOP) for alternate follow-up requirements.

Subjects who withdraw, or are withdrawn or terminated from the study, or are lost to follow-up after signing the informed consent form, randomization, and receipt of study vaccine will not be replaced. If subjects who withdraw, or are withdrawn or terminated from the study, or are lost to follow-up after signing the informed consent form and randomization but before receipt of study vaccine, additional subjects may be enrolled and randomized for replacement.

5.4.5. Termination of Study

Although the study Sponsor has every intention of completing the study, it reserves the right to terminate the study at any time for clinical or administrative reasons. Reasons for termination include, but are not limited to, study closure due to DSMB review and recommendation and at the discretion of DMID.

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6. STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1. Study Product Description

Seasonal Trivalent Inactivated Influenza Vaccine (IIV3)

Trivalent Inactivated Influenza Vaccine (IIV3) for intramuscular injection is indicated for active immunization against influenza disease caused by influenza virus subtypes A and type B present in the vaccine. IIV3 is manufactured using virus grown in eggs.

M-001

Multimeric-001 (M-001) vaccine developed by BiondVax is a multimeric peptide-based vaccine, a single chain recombinant protein containing 9 B and T cell epitopes derived from HA, NP and M1 sequences that are conservatively expressed among influenza A and B viruses.
It has been produced under aseptic conditions and according to the rules of current Good Manufacturing Practice and guidelines applicable to investigational medicinal products (IMPs). The vaccine lots used in this trial have been tested and released by the quality control department of the BiondVax.

Placebo (Normal Saline)

Sterile normal saline will be used as the placebo.

6.1.1. Acquisition

IIV3 will be purchased by DHHS.

M-001 will be provided by BiondVax under contract to DHHS.

Placebo (normal saline) will be provided by the DMID Clinical Agents Repository (CMS, Fisher BioServices).

IIV3, M-001 and normal saline will be provided through the DMID CMS to the participating VTEU site research pharmacies after all applicable regulatory and site approvals have been obtained. Should the site PI require additional study product during this trial, further instructions are provided in the protocol-specific MOP.

6.1.2. Formulation, Storage, Packaging, and Labeling

Seasonal Trivalent Inactivated Influenza Vaccine (IIV3)

The IIV3 is supplied as a sterile, clear, and colorless to slightly opalescent suspension in a single-dose, 0.5ml prefilled syringe. It is formulated to contain 45 mcg hemagglutinin (HA) per 0.5ml dose in the recommended ratio of 15 mcg HA for each of the three vaccine strains.

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It contains no preservative (i.e., thimerosal-free).

The vaccine must be stored at 2°C to 8°C (36°F to 46°F). Do not freeze.

M-001

M-001 is supplied as a sterile, cloudy-white suspension in single-dose vials containing:

- 1 mg M-001 per 0.4 mL (dose for injection)

Each vial contains a fill volume of 0.6 mL at a concentration of 2.5mg/mL. It contains no preservative (i.e., non-thimerosal).

The vials containing study product must be stored at 2°C to 8°C (35.6°F to 46.4°F). Do not freeze.

Placebo (Normal Saline)

0.9% Sodium Chloride, USP or normal saline is a sterile, nonpyrogenic, isotonic solution; each mL contains sodium chloride 9 mg. It contains no bacteriostatic agent, antimicrobial agent, preservatives, or added buffer and is supplied only in single-dose containers. The solution may contain hydrochloric acid and/or sodium hydroxide for pH adjustment (pH 5.3, range 4.5-7.0). The normal saline vials are to be stored at 20-25°C (68°F to 77ºF) [See USP Controlled Room Temperature; excursions between 15°C and 30°C (59°C and 86°F) are permitted].

Each study product will be labeled according to manufacturer specifications and include the statement "Caution: New Drug – Limited by Federal (or United States) Law to Investigational Use."

Further details are included in the Investigator's Brochures for the M-001 vaccine and the IIV3 Package Insert, as well as in the protocol-specific MOP.

6.1.3. Product Storage and Stability Procedures

The temperature of the storage unit must be manually recorded daily (excluding non-business days and holidays as applicable), monitored during the duration of this trial per the participating VTEU sites' standard operating procedures, and documentation will be maintained. If the temperature fluctuates outside of the required range, the affected study product(s) must be quarantined at the correct storage temperature and labeled as 'Do Not Use' (until further notice). If any study product appears to have been damaged, contaminated or discolored, contains visible particulate matter (for normal saline and IIV3 vaccine), or if there are any concerns regarding its integrity, do NOT use the affected study product(s). The pharmacist must alert the site PI and study coordinator, if the temperature fluctuates outside of the required range. The site PI or responsible person should immediately contact the DMID Product Support Team at DMIDProductSupportTeam@niaid.nih.gov for further instructions before any additional study vaccinations are administered. Based on the information collected, DMID and/or the manufacturer will determine whether the affected study product(s) can be used. If it cannot be used, the site will receive specific instructions on how to return the affected study product(s) to the DMID CAR or destroy it on site. Additional instructions for quarantine are provided in the protocol-specific MOP.

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6.2. Dosage, Preparation and Administration of Study Intervention/ Investigational Product

See the protocol-specific MOP for detailed information on the preparation, labeling, storage, and administration of study vaccine. Study vaccine preparation will be performed by the participating VTEU site's research pharmacist on the same day of study vaccine administration.

M-001 investigational influenza vaccine product:

The 1 mg of M-001 dose will be administered as a single 0.4 mL intramuscular injection.

Visually inspect M-001 vaccine upon receipt and prior to use. After gentle shaking, the suspension will be cloudy white in appearance. If the M-001 vaccine precipitated, it should be re-suspended by tapping on the vial with the finger or mixing by vortex until the suspension looks homogeneous. If the M-001 vaccine appears to have been damaged, contaminated or discolored, or if there are any concerns regarding its integrity, do NOT use the affected study product(s).

Additional instructions for quarantine and DMID contact information are provided in the protocol-specific MOP.

Seasonal trivalent inactivated influenza vaccine product:

The IIV3 will be administered as an intramuscular injection using a single-dose 0.5mL prefilled syringe.

Shake thoroughly and inspect visually before use. The vaccine product should be free of particulate matter and discoloration prior to administration. If either of these conditions exists, the vaccine should not be administered. Administer the vaccine immediately after the thorough shaking and inspection.

Normal Saline (Placebo):

The saline placebo dose will be administered as a single 0.4 mL intramuscular injection.

Visually inspect the placebo (normal saline) upon receipt and prior to use. The solution will be clear to colorless in appearance. If the placebo appears to have been damaged, contaminated or discolored, contain visible particulate matter, or if there are any concerns regarding its integrity, do NOT use the affected study product(s). Additional instructions for quarantine and DMID contact information are provided in the protocol-specific MOP.

Study vaccine administration will be performed by an unblinded study personnel member who is credentialed to administer medications/vaccines and may also participate in dose preparation. Each dose of study vaccine will be administered via a single IM injection given in the deltoid muscle of the subjects' preferred arm. The site of injection (right or left arm) will be recorded on the appropriate data collection form. Aseptic technique will be used for the withdrawal and

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administration of each dose of study vaccine using a disposable sterile needle appropriate in length for each subject and a disposable sterile 1-mL syringe. See the protocol-specific MOP for information on how to administer IM injections. Each dose of study vaccine must be administered within 30 minutes of drawing into the syringe, and the prepared syringe must be stored at room temperature until administered.

6.3. Modification of Study Intervention/Investigational Product for a Participant

Individuals must meet the appropriate inclusion and exclusion criteria prior to receiving the subsequently scheduled study injections. There will be no replacement of individuals who do not qualify to receive study doses 2 or 3. There will be no dose schedule modifications, other than within the protocol-specified windows. If a subject's study vaccination (study doses 2 or 3) is deferred, vaccination should be rescheduled to occur within the acceptable protocol-specified window for that visit. No exceptions to the protocol-specified window will be made.

Subjects who do not receive Dose 2 or Dose 3 will continue with follow-up safety assessments (may be conducted by phone call rather than in person) continuing through approximately 6 months after their last dose of M-001 or placebo. These subjects will also be asked to provide a venous blood sample for anti-viral antibody assays at approximately 21 days after their last dose of M-001. See Sections 5.4.3 and 5.4.4 for reasons for and handling of withdrawals and discontinuation of treatment. See the protocol-specific MOP for alternate follow-up requirements.

6.4. Accountability Procedures for the Study Intervention/ Investigational Product(s)

After receipt of the IIV3, M-001, and placebo vials, the site PI is responsible for study product distribution and disposition, and has ultimate responsibility for study product accountability. The Site PI may delegate to the site Research Pharmacist the responsibility for study product accountability. The site Research Pharmacist will be responsible for maintaining complete records and documentation of product receipt, accountability, dispensation, temperature and storage conditions, and final disposition of study product. All study product, whether administered or not, must be documented on the appropriate study product accountability record or dispensing log.

Used and unused vials of M-001 and placebo and IIV3 will be retained until monitored and released for disposition as applicable. Upon completion or termination of the study and after the final monitoring visit, final disposition of unused study products will be determined by DMID and communicated to the participating VTEU sites by the DMID Clinical Project Manager. For details regarding final disposition of study products see the Manual of Procedures (MOP).

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6.5. Assessment of Subject Compliance with Study Intervention/ Investigational Product

Study product will be administered to subjects by an unblinded vaccine administrator via IM injection at all dosing times according to subject treatment assignment and as described in Section 6.2. Thus, subject compliance is not anticipated to be an issue. Deviations from the dose schedule may only occur as described in Section 6.3.

6.6. Concomitant Medications/Treatments

Administration of any medications, therapies, or vaccines will be recorded on the appropriate data collection form. Concomitant medications will include all current medications, vaccinations and medications taken in the 60 days prior to Day 1 through approximately Day 43. Subjects who do not receive both M-001 study vaccinations will have concomitant medications collected through approximately 21 days after the first study vaccination, or early termination, whichever occurs first. Medications reported in the electronic case report form (eCRF) are limited to those taken within 30 days prior to the first study vaccination through approximately 21 days after the second M-001 study vaccination. Concomitant medications (vaccines only) will be recorded on the appropriate data collection form after Visit 7 through the end of the study. Prescription and over-the-counter drugs will be included as well as herbals, vitamins and supplements. In addition, receipt of any non-study vaccines will be solicited at each clinic visit or phone call, and reported in the eCRF.

Use of new medication should prompt evaluation for the presence of a new diagnosis of chronic medical disease or condition.

Medications that might interfere with the evaluation of the investigational product should not be used unless absolutely necessary. Medications in this category include the prohibited medications per the Subject Exclusion Criteria (see Section 5.2). In addition, the site PI or appropriate sub-I may identify other mediations that should not be used due to a risk to subject safety or assessment of reactogenicity and immunogenicity.

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7. STUDY SCHEDULE

7.1. Enrollment/Baseline (Visit 01, Day 1, Dose 1, Clinic Visit)

- Subjects will be provided with a description of the study (purpose and study procedures) and asked to read and sign the informed consent form (ICF). The ICF will be signed prior to performing any study procedures, including administration of the first study vaccination.
- Eligibility criteria will be reviewed with subjects prior to the first study vaccination. Subject receipt of licensed 2016-2017 and 2017-2018 seasonal influenza vaccine, what type (LAIV or inactivated), and approximate date of vaccination will be recorded on the appropriate data collection form, if known.
- Complete medical history will be obtained by interview of subjects prior to the first study vaccination to assure eligibility.
- All concomitant medications and vaccinations taken within 30 days prior to signing the ICF will be recorded on the appropriate data collection form prior to the first study vaccination.
- Vital signs, including oral temperature, pulse, and blood pressure, will be obtained prior to the first study vaccination. Vital signs assessed on Day 1 (Visit 01) prior to the first study vaccination will be considered as baseline. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- Height and weight will be collected on Day 1 prior to the first study vaccination for the calculation of Body Mass Index (BMI).
- A targeted physical examination may be performed prior to the first study vaccination by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-I, if indicated based on review of complete medical history.
- Counsel women of childbearing potential on contraception and avoidance of pregnancy.
- A urine or serum pregnancy test will be performed within 24 hours prior to the first study vaccination on all female subjects of childbearing potential. Results must be negative and known prior to randomization and first study vaccination.
- Approximately 10 mL of venous blood will be collected prior to the first study vaccination for safety labs.
- Approximately 10 mL of venous blood will be collected prior to the first study vaccination for baseline antibody assays.
- Approximately 60 mL of venous blood will be collected prior to the first study vaccination for isolation of PBMCs.
- Subjects will be enrolled in AdvantageEDC and randomly assigned to a treatment arm prior to the first study vaccination.

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- Pre-administration reactogenicity assessments will be performed prior to the first study vaccination to establish baseline. Subjects will then receive a single dose of study vaccine via IM injection in the deltoid muscle of the preferred arm. The site of injection (right or left arm) and time of administration will be recorded on the appropriate data collection form. Subjects will be observed in the clinic for at least 20 minutes after the first study vaccination. The study vaccination site will be examined, and any AE/SAEs will be assessed and recorded on the appropriate data collection form prior to discharge from the clinic.
- Subjects will be provided with a Memory Aid and other study-related materials to record daily oral temperature, solicited injection site and systemic reactions, any unsolicited AEs, and concomitant medications/vaccinations. Subjects will be encouraged to take their temperature around the same time each day for 8 days post vaccination. Subjects will be instructed on how to use the Memory Aid and how to measure and record AEs prior to discharge from the clinic. Subjects will be instructed to notify the study center if they develop any severe reactions after the first study vaccination. If the site PI or appropriate sub-I deems the reaction severe enough, s/he will give further instructions on the proper course of action, including a return to the clinic for immediate evaluation if appropriate.

7.2. Follow-up

7.2.1. Visit 2, Day 9, Clinic Visit (8 days [+2 days] post first study vaccination)

- Study personnel will review the Memory Aid information with subjects and assess and record all AE/SAEs and concomitant medications on the appropriate data collection form.
- Counsel women of childbearing potential on contraception and avoidance of pregnancy.
- If indicated by review of Memory Aid, obtain vital signs.
- A targeted physical examination may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-I, if indicated based on review of interim medical history.
- Examine the vaccination site for Dose 1.
- Approximately 10 mL of venous blood will be collected for safety labs.

7.2.2. Visit 3, Day 15, Clinic Visit (14 days [+2 days] post first study vaccination)

- Study personnel will review interim medical history with subjects and assess and record all AE/SAEs and concomitant medications on the appropriate data collection form.
- Counsel women of childbearing potential on contraception and avoidance of pregnancy.

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- If indicated, obtain vital signs.
- A targeted physical examination may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-I, if indicated based on review of interim medical history.
- Approximately 40 mL of venous blood will be collected for isolation of PBMCs.

7.2.3. Visit 4, Day 22, Dose 2, Clinic Visit (21 days [+2 days] post first study vaccination)

- Eligibility criteria will be reviewed with subjects prior to the second study vaccination to assure continued eligibility.
- Obtain interim medical history by interview of subjects prior to the second study vaccination and note any changes since the previous visit.
- All concomitant medications will be recorded on the appropriate data collection form prior to the second study vaccination.
- All AE/SAEs will be assessed and recorded on the appropriate data collection form prior to the second study vaccination.
- Vital signs, including oral temperature, pulse, and blood pressure, will be obtained prior to the second study vaccination. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- A targeted physical examination may be performed prior to the second study vaccination by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-I, if indicated based on review of interim medical history.
- Counsel women of childbearing potential on contraception and avoidance of pregnancy.
- A urine or serum pregnancy test will be performed within 24 hours prior to the second study vaccination on all female subjects of childbearing potential. Results must be negative and known prior to the second study vaccination.
- Approximately 10 mL of venous blood will be collected prior to the study vaccination for antibody assays.
- Pre-administration reactogenicity assessments will be performed prior to the second study vaccination to establish baseline. Subjects will then receive a single dose of study vaccine via IM injection into the deltoid muscle of the same arm that received Dose 1 as long as there is no interference with the reactogenicity assessment. The site of injection (right or left arm) and time of administration will be recorded on the appropriate data collection form. Subjects will be observed in the clinic for at least 20 minutes after the second study vaccination. The study vaccination site for Dose 2 will be examined, and any AE/SAEs will be assessed

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and recorded on the appropriate data collection form prior to discharge from the clinic.

- Subjects will be provided with a Memory Aid and other study-related materials to record daily oral temperature, solicited injection site and systemic reactions, any unsolicited AEs, and concomitant medications. Subjects will be encouraged to take their temperature around the same time each day. Subjects will be instructed on how to use the Memory Aid and how to measure and record AEs prior to discharge from the clinic. Subjects will be instructed to notify the study center if they develop any severe reactions after the second study vaccination. If the site PI or appropriate sub-I deems the reaction severe enough, s/he will give further instructions on the proper course of action, including a return to the clinic for immediate evaluation if appropriate.

7.2.4. Visit 5, Day 30, Clinic Visit (8 days [+2 days] post second study vaccination)

- Study personnel will review the Memory Aid information with subjects and assess and record all AEs/SAEs and concomitant medications on the appropriate data collection form.
- Counsel women of childbearing potential on contraception and avoidance of pregnancy.
- If indicated, obtain vital signs.
- A targeted physical examination may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-I, if indicated based on review of interim medical history.
- Examine the vaccination site for Dose 2.
- Approximately 10 mL of venous blood will be collected for safety labs.

7.2.5. Visit 6, Day 36, Clinic Visit (14 days [+2 days] post second study vaccination)

- Study personnel will review interim medical history with subjects and assess and record all AE/SAEs and concomitant medications on the appropriate data collection form.
- Counsel women of childbearing potential on contraception and avoidance of pregnancy.
- If indicated, obtain vital signs.
- A targeted physical examination may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-I, if indicated based on review of interim medical history.
- Approximately 40 mL of venous blood will be collected for isolation of PBMCs.

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7.2.6. Visit 7, Day 43, Clinic Visit (21 days [+7 days] post second study vaccination)

- Study personnel will review interim medical history with subjects and assess and record all AE/SAEs and concomitant medications on the appropriate data collection form.
- If indicated, obtain vital signs.
- A targeted physical examination may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-I, if indicated based on review of interim medical history.
- Counsel women of childbearing potential on contraception and avoidance of pregnancy.
- Approximately 10 mL of venous blood will be collected for influenza antibody assays.
- Approximately 60 mL of venous blood will be collected for isolation of PBMCs.

7.2.7. Visit 8, Day 78, Clinic Visit (56 days [+7 days] post second study vaccination)

- Study personnel will review interim medical history with subjects and assess and record all SAEs on the appropriate data collection form.
- Concomitant medications (vaccines only) will be recorded on the appropriate data collection form.
- Counsel women of childbearing potential on contraception and avoidance of pregnancy through study day 82 (60 days after second vaccination).
- If indicated, obtain vital signs.
- A targeted physical examination may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-I, if indicated based on review of interim medical history.
- Approximately 10 mL of venous blood will be collected for influenza antibody assays.
- Approximately 40 mL of venous blood will be collected for isolation of PBMCs.

7.2.8. Visit 9, Day 172, Dose 3 (150 days [±30 days] post second study vaccination)

- Eligibility criteria will be reviewed with subjects prior to the third study vaccination IIV3) to assure continued eligibility.
- Obtain interim medical history by interview of subjects.
- Concomitant medications (vaccines only) will be recorded on the appropriate data collection form.

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- All SAEs will be assessed and recorded on the appropriate data collection form.
- Vital signs, including oral temperature, pulse, and blood pressure, will be obtained prior to the third study vaccination. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- A targeted physical examination may be performed prior to the third study vaccination by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-I, if indicated based on review of interim medical history.
- Counsel women of childbearing potential on contraception and avoidance of pregnancy.
- A urine or serum pregnancy test will be performed within 24 hours prior to the third study vaccination on all female subjects of childbearing potential. Results must be negative and known prior to the third study vaccination.
- Approximately 20 mL of venous blood will be collected prior to the third study vaccination for influenza antibody assays.
- Approximately 40 mL of venous blood will be collected prior to the study vaccination for isolation of PBMCs.
- Subjects will then receive a single dose of IIV3 study vaccine via IM injection into the deltoid muscle of the same arm that received Dose 1. The site of injection (right or left arm) and time of administration will be recorded on the appropriate data collection form.
- Subjects will be instructed to notify the study center if they develop any worrisome adverse events or experienced an SAE.

7.2.9. Visit 10, Day 186, Clinic Visit (14 days [+3 days] post third study vaccination)

- Study personnel will review interim medical history and assess and record all SAEs.
- Concomitant medications (vaccines only) will be recorded on the appropriate data collection form.
- Counsel women of childbearing potential on contraception and avoidance of pregnancy.
- If indicated, obtain vital signs.
- A targeted physical examination may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-I, if indicated based on review of interim medical history.
- Approximately 40 mL of venous blood will be collected for isolation of PBMCs.

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7.3. Study Visit 11, Day 200 Clinic Visit (28 days +7 post third study vaccination)

7.3.1. Visit 11 activities

- Study personnel will review interim medical history with subjects and assess and record all SAEs on the appropriate data collection form.
- Concomitant medications (vaccines only) will be recorded on the appropriate data collection form.
- If indicated, obtain vital signs.
- A targeted physical examination may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-I, if indicated based on review of interim medical history.
- Approximately 20 mL of venous blood will be collected for influenza antibody assays.
- Approximately 40 mL of venous blood will be collected for isolation of PBMCs.

7.3.2. Study Visit 12, Day 200 (+14 days), Safety Follow-up Phone Call (to be Performed Only If Visit 11 Occurs Before Study Day 200)

- Study personnel will review interim medical history with subjects and assess and record all SAEs on the appropriate data collection form.
- Concomitant medications (vaccines only) will be recorded on the appropriate data collection form.

7.4. Early Termination Visit

The following activities will be performed at the early termination visit for subjects who withdraw,
or are withdrawn or terminated from the study:

- Obtain interim medical history by interview of subjects and note any changes since the previous visit.
- Memory Aid information will be reviewed with subjects (if within 8 days after a M-001 study vaccination).
- If indicated by medical history, obtain vital signs. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- Review and record all concomitant medications (if prior to 21 days after the last study vaccination).

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- Information regarding AEs/SAEs will be recorded on the appropriate data collection form. AEs will be limited to SAEs if after 21 days following the second M-001 study vaccination.

- A targeted physical examination, including an assessment for signs suggestive of a PIMMC, may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-I, if indicated based on review of interim medical history.

- Examine study vaccination site, and perform post-administration reactogenicity assessment (if within 8 days after a M-001 study vaccination).

- Approximately 10 mL of venous blood will be collected for clinical labs, and performed by the central (clinical) laboratory (if within 7 (+2) days after a M-001 study vaccination), or if indicated based on review of interim medical history, or to follow-up on any grade 2 or higher laboratory abnormalities.
- Approximately 10 mL of venous blood will be collected for influenza antibody assays.
- Approximately 40 mL of venous blood will be collected for isolation of PBMCs.

7.5. Unscheduled Visit

Unscheduled visits may occur at any time during the study. Any of the following activities may be performed:

- Review Memory Aid (if within 8 days after the previous study vaccination, inclusive of vaccination day).
- Review and record all concomitant medications (if prior to 21 days after the last study vaccination).
- All AEs/SAEs will be recorded on the appropriate data collection form. AEs will be limited to SAEs if after 21 days after the last study vaccination.
- Obtain interim medical history by interview of subjects and note any changes since the previous visit (if indicated).
- If indicated, obtain vital signs. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- A targeted physical examination may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-I, if indicated based on review of interim medical history.

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- Examine study vaccination site and perform post- administration reactogenicity assessment (if within 8 days after the last study vaccination, inclusive of vaccination day).

- Approximately 10 mL of venous blood will be collected for clinical labs, and performed by the central (clinical) laboratory (if within 9 days after a M-001 study vaccination), or if indicated based on review of interim medical history, or to follow-up on any laboratory abnormalities determined to be AEs.

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8. STUDY PROCEDURES/EVALUATIONS

8.1. Clinical Evaluations

Medical History: Will be obtained by interview of the subjects. Subjects will be queried regarding a history of significant medical disorders of the head, eyes, ears, nose, throat, mouth, cardiovascular system, lungs, gastrointestinal tract, liver, pancreas, kidney, nervous system, blood, lymph glands, endocrine system, musculoskeletal system, skin, and genital/reproductive tract. A history of any allergies, cancer, immunodeficiency, psychiatric illness, and substance abuse will be solicited. The collection of medical history information will include a review of vaccine history and plans for vaccinations.

Concomitant Medications: All current medications and medications taken in the 30 days before Study Day 1 (prescription and over-the-counter drugs) will be included, as well as vaccinations, vitamins and supplements, through 21 days after the second M-001 study injection. In addition, all current medications and medications and vaccinatins taken in the 30 days before Study Day 172 (Visit 09) (prescription and over-the-counter drugs) will be included, as well as vaccinations, vitamins and supplements, through 21 days after the IIV3 study injection. Assessment of eligibility also will include a review of prohibited medications (per the exclusion criteria).

Vitals signs: Blood pressure, oral temperature, and pulse will be collected at each of the study visits when a study vaccination is scheduled [Day 1 (Visit 01), Day 22 (Visit 04), Day 172 (Visit 09), and as needed at the other study visits.

Height, weight: These parameters will be measured at Day 1 (Visit 01).

Targeted Physical Examination: This may be conducted at any study visit based on indicated symptoms.

Reactogenicity Assessments: This will include an assessment of solicited AEs occurring from the time of each study vaccination [Day 1 (Visit 01), Day 22 (Visit 04), through 7 days after M-001study vaccinations (inclusive of vaccination day), which includes an assessment of erythema/redness, induration/swelling, pain, tenderness, ecchymosis and pruritus at the injection site; fever, chills, fatigue, malaise, body aches (exclusive of injection site), arthralgia (exclusive of injection site), headache, and nausea.

Memory Aids: All subjects will complete a subject Memory Aid from the time of each study vaccination [Day 1 (Visit 01) and Day 22 (Visit 04) through 7 days after the M-001 study vaccination. Subject Memory Aids will be reviewed with the subject for AEs at the clinic visit following each study injection. If a subject noted ongoing injection site or systemic reactogenicity on the 7th day following the study injection, the Memory Aid will continue to be reviewed until resolved. The Memory Aids will be discarded after review.

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8.2. Laboratory Evaluations

Schedules and volumes of clinical laboratory tests and immunogenicity assays are specified in Appendix A.

8.2.1. Clinical Laboratory Evaluations

Urine or serum pregnancy tests will be performed by the local or site laboratory within 24 hours prior to each study vaccination (Day 1 (Visit 01) and approximately Day 22 (Visit 04), and Day 172 (Visit 09)) on all female subjects of childbearing potential. Results must be negative and known prior to randomization on Day 1 (Visit 01) and administration of each study vaccination to be eligible for participation in the study and receipt of each dose of study vaccine, respectively.

Clinical safety laboratory parameters to be evaluated after receipt of the first two doses of study vaccine will include: WBC, hemoglobin, platelets, ALT, total bilirubin, and creatinine. These evaluations will be performed by the central (clinical) laboratory. Venous blood samples (approximately 10 mL) will be collected from each subject prior to vaccination at baseline (Visit 01) and approximately 8 days after Dose 1 and Dose 2 of study vaccination (Visits 02 and 05, respectively).

8.2.2. Special Assays or Procedures

<u>HAI and Neut Antibody</u>

Assays to determine serum levels of HAI and Neut antibodies will be performed at St Louis University. Subjects who withdraw early will have HAI and Neut antibody assays run on available sera.

<u>M-001 Antibody</u>

Assays to determine serum levels of antibodies to M-001 will be performed by BiondVax. Subjects who withdraw early will have M-001 antibody assays run on available sera.

<u>Cellular Studies</u>

Assays to measure T cell responses will be performed at Baylor College of Medicine.

8.2.3. Specimen Preparation, Handling, and Shipping

8.2.3.1. Instructions for Specimen Preparation, Handling, and Storage

Instructions for specimen preparation, handling, and storage are included in the protocol-specific MOP, as appropriate.

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8.2.3.2. Specimen Shipment

Specimen shipment will occur at intervals during the course of this study following all applicable International Air Transport Association (IATA) requirements and according to the specifics for storage temperature and documentation as detailed in the central (clinical) laboratory manual and protocol-specific MOP as appropriate.

Sera and PBMCs will be shipped from the participating VTEU sites to the DMID CAR. Sera for HAI and neut assays will then be provided by the DMID CAR to St Louis University in a blinded manner once they become available to the DMID CAR. Serum samples for detection of antibody responses to M-001 will be provided to BiondVax by the DMID CAR in a blinded fashion once they become available. PBMC samples will be provided by the DMID CAR to Baylor College of Medicine for the planned cellular assays.

Further instructions for specimen shipment are included in the central clinical laboratory manual and the protocol-specific MOP, as appropriate.

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9. ASSESSMENT OF SAFETY

9.1. Specification of Safety Parameters

Safety will be assessed by the frequency and severity of:

1. All SAEs occuring from the time of the first study vaccination through the end of the study
2. Solicited AEs – reactogenicity events occurring on the day of each study vaccination through 7 days* after each M-001 study vaccination:

   a) Injection site reactions including pruritus, ecchymosis, erythema, induration/swelling, pain, and tenderness.

   b) Systemic reactions including fever, feverishness, fatigue, malaise, myalgia, arthralgia, headache, and nausea.

3. Unsolicited AEs – non-serious AEs occurring from the time of the first M-001 study vaccination through approximately 21 days after the second M-001 study vaccination.

4. Safety laboratory abnormalities occurring from the time of the first study vaccination through 8 days after the last dose of M-001.

   a) Clinical safety laboratory parameters measured: WBC, hemoglobin, platelets, ALT, total bilirubin, and creatinine

9.2. Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1. Adverse Events

Adverse Event (AE): International Conference on Harmonisation (ICH) E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

AEs, including solicited injection site and systemic (subjective and quantitative) reactions, not meeting the protocol-defined criteria for SAEs will be captured on the appropriate data collection form and electronic case report form (eCRF). Information to be collected for unsolicited AEs includes event description, date of onset, licensed study physician's assessment of severity and relationship to study product and alternate etiology (if not related to study product) (assessed only by those with the training and authority to make a diagnosis and listed on the Form FDA

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1572 as the site PI or sub-I), date of resolution of the event, seriousness and outcome. AEs while on study will be documented appropriately regardless of relationship. AEs will be followed to resolution.

Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if the severity of any pre-existing medical condition increases, it will be recorded as an AE.

AEs must be graded for severity and assessed for relationship to study product (see definitions below). AEs characterized as intermittent require documentation of onset and duration of each episode. The start and stop date of each reported AE will be recorded on the appropriate data collection form and eCRF.

FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Severity of Event: AEs will be assessed by a licensed study physician listed on the Form FDA 1572 as the site PI or appropriate sub-I using a protocol-defined grading system (see Section 9.2.2). For events not included in the protocol-defined grading system, the following guidelines will be used to quantify severity:

- Mild (Grade 1): Events require minimal or no therapeutic measure(s) and do not interfere with the subject's usual daily activities.
- Moderate (Grade 2): Events result in a low level of inconvenience or concern with therapeutic measures. Moderate events cause some interference with functioning and usual daily activities.
- Severe (Grade 3): Events interrupt the subject's usual daily activities and therapeutic measure(s) indicated. Severe events are usually incapacitating.

Relationship to Study Product: The study physician's assessment of an AE's relationship to study product is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. The relationship to study product must be assessed for AEs using the terms: related or not related. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used:

- Related – There is a reasonable possibility that the study product caused the AE. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the AE.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

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9.2.2. Reactogenicity

Reactogenicity events are AEs that are common and known to occur following administration of this type of study vaccine. The following Toxicity Grading Scales will be used to grade solicited injection site and systemic (subjective and quantitative) reactions:

Table 2: Injection Site Reactogenicity Grading

Injection Site Reaction	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Pain – experienced without touching the injection site (spontaneous discomfort)	Subject is aware of pain, but it does not interfere with daily activity, **and** no pain medication is taken	Subject is aware of pain; there is interference with daily activity **or** it requires repeated use of a non-narcotic pain reliever for >24 hours	Subject is aware of pain, **and** it prevents daily activity or requires any use of a narcotic pain reliever
Tenderness – hurts only when injection site is touched or the arm is moved	The area immediately surrounding the injection site hurts only when touched or with arm motion, **and** it does **not** interfere with daily activity	The area immediately surrounding the injection site hurts when touched or with arm motion, **and** it interferes with daily activity	The area immediately surrounding the injection site hurts when touched or with arm motion, **and** it prevents daily activity
Pruritus	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity
Ecchymosis*	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity
Erythema*	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity
Induration/Edema*	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity

* Will be also measured in mm but size will not be used as halting criteria.

Ecchymosis, erythema, and induration /edema as analyzed by measurement will be graded as follows:

Table 3: Injection Site Reactogenicity Measurements

Injection Site Reaction	Small	Medium	Large
Ecchymosis *	<20 mm	20 mm – 50 mm	>50 mm
Erythema*	<20 mm	20 mm – 50 mm	>50 mm
Induration /Edema*	<20 mm	20 mm – 50 mm	>50 mm

* Will not be used as halting criteria.

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Table 4: Subjective Systemic Reactogenicity Grading

Systemic (Subjective)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Feverishness (Chills/Shivering/Sweating)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Fatigue	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Malaise	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Myalgia*	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Arthralgia*	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Headache	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Nausea	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity

* Not at injection site.

Oral temperature# will be graded as follows:

Table 5: Quantitative Systemic Reactogenicity Grading

Systemic (Quantitative)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Fever* - oral†	38.0°C – 38.4°C 100.4°F – 101.1°F	38.5°C – 38.9°C 101.2°F – 102.0°F	>38.9°C >102.0°F

Oral temperature assessed on Day 1 prior to the first primary series study vaccination will be considered as baseline.

* A fever can be considered not related to the study product if an alternative etiology can be documented.

† Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

9.2.3. Additional Adverse Event Severity Grading

Pulse and blood pressure# will be graded as follows:

Table 6: Pulse and Blood Pressure Grading

Physiologic Parameter	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Bradycardia - beats per minute	45 – 49	40 – 44	<40
Tachycardia - beats per minute	116 – 130	131 – 155	>155
Hypotension (systolic) mm Hg	80 – 84	75 – 79	<75
Hypotension (diastolic) mm Hg	50 – 54	45 – 49	<45

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Physiologic Parameter	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Hypertension (systolic) mm Hg	151 – 155	156 – 160	>160
Hypertension (diastolic) mm Hg	96 – 100	101 – 105	>105

[#] Pulse and blood pressure assessed on Day 1 prior to the first study vaccination will be considered as baseline.

Clinical safety laboratory results[#] will be graded as follows:

Table 7: Clinical Safety Laboratory Adverse Event Grading (28)

Hematology	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
WBC $10^3/\mu L$ (Decrease)	2.5 – 3.9	1.5 – 2.4	<1.5
WBC $10^3/\mu L$ (Increase)	10.6 – 15.0	15.1 – 20.0	>20.0
Hgb g/dL (Decrease) (Female)	10.1 – 11.4	8.5 – 10	<8.5
Hgb g/dL (Decrease) (Male)	11.0 – 12.4	9.5 – 10.9	<9.5
Platelets $10^3/\mu L$ (Decrease)	125 – 139	100 – 124	<100
Platelets $10^3/\mu L$ (Increase)	416 – 550	551– 750	>750

Chemistry	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
ALT IU/L (Increase) (Female)	44 – 100	101 – 200	>200
ALT IU/L (Increase) (Male)	61 – 138	139 – 275	>275
Total Bilirubin mg/dL (Increase) – when accompanied by any increase in ALT	1.3 – 1.5	1.6 – 1.8	>1.8
Total Bilirubin mg/dL (Increase) – when ALT is normal	1.3 – 1.8	1.9 – 2.4	>2.4
Creatinine mg/dL (Increase) (Female)	1.11 – 1.70	1.71 – 2.00	>2.00
Creatinine mg/dL (Increase) (Male)	1.38 – 1.70	1.71 – 2.00	>2.00

[#] Clinical laboratory evaluations assessed at the enrollment visit (Visit 01) will be considered as baseline.

9.2.4. Serious Adverse Events

An AE or suspected AE is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- death,
- a life-threatening AE*,

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- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.
- important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.
- all events described as Guillain-Barré syndrome will also be considered SAEs

* Life-threatening AE. An AE is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.

All SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 such as the site PI or sub-I.
- Recorded on the appropriate SAE CRF.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572.
- Reviewed and evaluated by an Independent Safety Monitor (ISM), the DSMB (periodic review unless related), DMID, and the IRB.

9.2.5. Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

The site PI or appropriate sub-I is responsible for reporting all AE/SAEs that are observed or reported during the study, regardless of the relationship to study product. AE/SAEs, abnormal clinical laboratory test values, or abnormal clinical findings will be documented, reported, and followed appropriately.

9.3. Reporting Procedures

Solicited injection site and systemic reactogenicity events will be documented from the time of each study vaccination (Day 1 (Visit 01) and approximately Day 22 (Visit 04)) through 7 days after each study vaccination.

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Unsolicited AEs will be documented from the time of the first study vaccination (Day 1 (Visit 01)) through approximately 21 days after the second M-001 study vaccination (approximately Day 43 (Visit 07).

SAEs will be documented from the time of the first study vaccination (Day 1 (Visit 01)) through the end of the study.

9.3.1. Serious Adverse Events

Any AE that meets a protocol-defined serious criterion must be submitted immediately (within 24 hours of site awareness) on an SAE form to the DMID Pharmacovigilance Group at the following address:

DMID Pharmacovigilance Group

Clinical Research Operations and Management Support (CROMS)

6500 Rock Spring Dr. Suite 650

Bethesda, MD 20817, USA

SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)

SAE FAX: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)

SAE Email Address: PVG@dmidcroms.com

In addition to the SAE form, selected SAE data fields must also be entered into AdvantageEDC. Please see the protocol-specific MOP for details regarding this procedure.

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible.

The site will send a copy of the SAE report(s) to the ISM when they are provided to the DMID Pharmacovigilance Group. The DMID Medical Monitor and DMID Clinical Project Manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID Medical Monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the site PI or appropriate sub-I becomes aware of an SAE that is suspected to be related to study product, the site PI or appropriate sub-I will report the event to the DMID Pharmacovigilance Group.

9.3.2. Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Following notification from the site PI or appropriate sub-I, DMID, the Investigational New Drug (IND) sponsor, will report any suspected adverse reaction that is both serious and unexpected. DMID will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the AE. DMID will notify FDA and all

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participating site PIs (i.e., all PIs to whom the sponsor is providing drug under its IND(s) or under any PI's IND(s)) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. DMID will also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. Relevant follow up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

All serious events designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

9.3.3. Reporting of Pregnancy

Pregnancies occurring in study subjects will be reported via AdvantageEDC on the Pregnancy Case Report form. No further study vaccinations will be administered to pregnant subjects, but, with the subject's permission, all study mandated blood samples will be obtained and the subject will continue in follow-up for safety events. Efforts will be made to follow all pregnancies reported during the course of the study to pregnancy outcome pending the subject's permission.

9.4. Type and Duration of Follow-up of Subjects after Adverse Events

AEs will be followed from the time of the first study vaccination (Day 1 (Visit 01)) through approximately 21 days after the second M-001 study vaccination (approximately Day 43 (Visit 07)

SAEs will be followed from the time of the first study vaccination (Day 1 (Visit 01)) through resolution even if this extends beyond the study-reporting period (end of study)). Resolution of an AE/SAE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

Follow-up procedures, evaluations, and outcomes will be recorded on the appropriate data collection form.

9.5. Halting Rules

Further enrollment and study vaccinations will be halted for DSMB review/recommendation if any of the following are reported:

- Any subject experiences ulceration, abscess, or necrosis at the injection site related to study product administration.

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- Any subject experiences laryngospasm, bronchospasm, or anaphylaxis within 1 day after administration of study product that is considered related to study product.

- Two or more subjects experience generalized urticaria within 3 days after administration of study product that is considered related to study product.

- Any subject experiences a study vaccine-related SAE from the time of the first study vaccination through the subject's last study visit.

- Any subject experiences acute weakness of limbs and/or cranial nerve innervated muscles (description of potential signal of GBS) after administration of study product.

This study will also be halted for DSMB review/recommendation if, within 8 days after administration of either of the first 2 vaccine doses of M-001 or placebo, any of the following occurs:

- 5% or more of subjects (with a minimum of 3 subjects) who received at least one dose of study vaccine to date experience the same severe (Grade 3) study vaccine-related injection site reaction. Ecchymosis (bruising), erythema (redness), and induration (hardness)/edema(swelling) will also be measured in mm but size will not be used as halting criteria.

- 5% or more of subjects (with a minimum of 3 subjects) who received at least one dose of study vaccine to date experience the same severe (Grade 3) study vaccine-related subjective systemic reaction, for which the severity (grade) is corroborated by study personnel.

- 5% or more of subjects (with a minimum of 3 subjects) who received at least one dose of study vaccine to date experience the same severe (Grade 3) study vaccine-related quantitative systemic reaction.

- 5% or more of subjects (with a minimum of 3 subjects) who received at least one dose of study vaccine to date experience the same severe (Grade 3) study vaccine-related clinical safety laboratory AE.

Grading scales for solicited injection site and systemic (subjective and quantitative) reactions are included in Section 9.2.2.

If any of the halting rules are met following any subject receipt of any study vaccination, then this study will not continue with the remaining enrollments or study vaccinations without a review by and recommendation from the DSMB to proceed.

DMID retains the authority to suspend additional enrollment and study interventions/ administration of study product during the entire study, as applicable.

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9.6. Safety Oversight (ISM plus DSMB)

9.6.1. Independent Safety Monitor (ISM)

The ISM is a physician with relevant expertise whose primary responsibility is to provide independent safety monitoring in a timely fashion. The ISM will review SAEs and other AEs as needed and provide an independent assessment to DMID. Each participating study site will have an ISM with experience in infectious diseases or internal medicine, in close proximity to the participating VTEU site, and have the authority to readily access study participant records.

9.6.2. Data and Safety Monitoring Board (DSMB)

Safety oversight will be conducted by a DSMB that is an independent group of experts that monitors subject safety and advises DMID. The DSMB members will be separate and independent of study personnel participating in this trial and should not have scientific, financial or other conflict of interest related to this trial. The DSMB will consist of members with appropriate expertise to contribute to the interpretation of the data from this trial.

The DSMB will review study progress and participant, clinical, safety, and reactogenicity data at the following time points. A review of immunogenicity data will be performed as specified below.

- Data review for safety at study-specific time frames; at least annually.
- After all 8-day post second study vaccination safety data are available for all study participants.
- Final review meeting: 6 to 8 months after clinical database lock to review the cumulative unblinded safety and immunogenicity data for this trial. The data will be provided in a standard summary format. The DSMB may be asked to provide recommendations in response to questions posed by DMID.
- Ad hoc when a halting rule is met, for immediate concerns regarding observations during this trial, or as needed.

The DSMB will operate under the rules of a DMID-approved charter that will be written at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. Procedures for DSMB reviews/meetings will be defined in the charter. The DSMB will review applicable data to include, but not limited to, study progress and participant, clinical, safety, and reactogenicity data. Reports may include enrollment and demographic information, medical history, concomitant medications, physical assessments, clinical laboratory values, dosing compliance, and solicited and unsolicited AE/SAEs. Additional data may be requested by the DSMB, and interim statistical reports may be generated as deemed necessary and appropriate by DMID. The DSMB may receive data in aggregate and presented by treatment arm. The DSMB may also be provided with expected and observed rates

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of the expected AEs in an unblinded fashion, and may request the treatment arm be unblinded for an individual subject if required for safety assessment. The DSMB will review grouped and unblinded data in the closed session only. The DSMB will meet and review this data at scheduled time points or ad hoc as needed during this trial as defined in the DSMB charter. As an outcome of each review/meeting, the DSMB will make a recommendation as to the advisability of proceeding with study vaccinations (as applicable), and to continue, modify, or terminate this trial.

Additionally, SDCC web reports using data entered into AdvantageEDC will be monitored to determine if any of the halting rules described in Section 9.5 are met.

If any of the halting rules are met, the study will not proceed with the remaining enrollment or study injections without a review by and recommendation from the DSMB to proceed.

Upon completion of this review and receipt of the advice of the DSMB, DMID will determine if study entry or study dosing should be interrupted or if study entry and study dosing may continue according to the protocol.

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10. CLINICAL MONITORING

10.1. Site Monitoring Plan

Site Monitoring will be conducted to ensure that human subject protection, study procedures, laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, CGP/ICH, and regulatory guidelines, and that the study is conducted in accordance with the protocol and sponsor SOPs. DMID, the sponsoring agency, or its designee will conduct site monitoring visits as detailed in the monitoring plan or in the Manual of Procedures.

Site visits will be made at standard intervals as defined by DMID and may be made more frequently as directed by DMID. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, case report forms, informed consent forms, medical and laboratory reports, and protocol compliance. Study monitors will meet with investigators to discuss any problems and actions to be taken and document visit findings and discussions.

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11. STATISTICAL CONSIDERATIONS

11.1. Study Hypotheses

The study is not designed to test a specific hypothesis for the primary safety objectives. Nevertheless, this trial represents an important opportunity to extend the M-001 safety base.

The primary immunogenicity objective is to determine whether priming with M-001 improves T cell responses. The hypothesis that will be tested is:

H_0: $GMP_M/GMP_c = 1 \rightarrow \log_{10}(GMP_M)-\log(GMP_c) = 0$
H_1: $GMP_M/GMP_c \neq 1 \rightarrow \log_{10}(GMP_M)-\log(GMP_c) \neq 0$

Where GMP_M and GMP_c are the geometric mean percentage (GMP) percentage of T cell subsets expressing perforin, CD107a, INFg, TNF-a, and IL-2 for the M-001 and control (placebo) treatment arms, respectively.

11.2. Study Outcome Measures

Please refer to Section 3.2.

11.3. Sample Size Considerations

11.3.1. Study Design

This is a Phase II, randomized, double-blind, controlled trial in 120 males and non-pregnant females, 18 to 49 years old, inclusive, who are in good health and meet all eligibility criteria. Subjects will be assigned randomly to 1 of 2 treatment arms (60 subjects per treatment arm) to receive two doses of M-001 or two placebo controls, followed by IIV3 (see Table 1). All doses will be administered IM approximately 21 days apart.

Reactogenicity will be measured by the occurrence of solicited injection site and systemic reactions from the time of each M-001 study vaccination through 8 days after each study vaccination (inclusive of vaccination day). Unsolicited non-serious AEs will be collected from the time of each M-001 study vaccination through approximately 21 days after each M-001 study vaccination. SAEs will be collected from the time of the first study vaccination through end of the study. Clinical laboratory evaluations for safety will be performed on venous blood collected approximately 8 days after each M-001 study vaccination (inclusive of vaccination day).

Immunogenicity testing will be performed as described in Section 8.2.2.

11.3.2. Study Population

The study population for this clinical trial includes 120 males and non-pregnant females, 18 to 49 years old, inclusive, who are in good health and meet all eligibility criteria. The subjects will be

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recruited from the general population at the participating VTEU sites that have substantial experience conducting large influenza vaccine studies.

11.3.3. Subject Enrollment and Follow-up

Based on the accrual rate for similar studies, it seems reasonable to expect that the participating VTEUs will be able to enroll this trial in a timely fashion. Prior experience suggests up to 5% of subjects may be excluded from the per protocol analysis for the primary immunogenicity outcome either because they were lost-to-follow-up or otherwise do not have data available following the second study vaccination or because they had a protocol deviation requiring their exclusion from the per protocol analysis.

11.3.4. Sample Size

This study is planned to enroll 120 subjects (60 per treatment arm). The following illustrates the precision and power that is available for select estimates and comparisons of interest.

Table 8 indicates the probability of observing one or more safety events, such as solicited injection site or systemic reactogenicity events or an unsolicited non-serious AE of a particular type, for a single treatment arm (N=60) and all enrolled subjects (N=120).

Table 8: Power (%) to Detect Safety Events

Event Frequency	Single Treatment Arm N=60	Two Treatment Arms N=120
≥10% Very Common	99.8	>99.9
≥1% Common	45.3	70.1
≥0.1% Uncommon	5.8	11.3
≥0.01% Rare	<1	1.2

Binomial confidence intervals (CI) are widest (have the least precision) when the response rate is 50%. Table 9 is presented to indicate the worst case scenario for precision of observed exact (Clopper-Pearson) binomial confidence intervals.

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Table 9: Precision of Binomial Confidence Intervals:

	N	95% CI
Single Treatment Arm	60	(36.8,63.2)
Two Treatment Arms	120	(40.7, 59.3)

With N = 60 subjects per arm and assuming the standard deviation of logGMP is 0.75, the study will have 80% power to detect a 2.5-fold increase in the GMP of T cell subsets expressing IFN-γ. Table 10 illustrates a power analysis considering a range of standard deviations for log GMP and a range of GMP fold-change with N=60 subjects per am, and further includes the power under the conservative assumption of 10% drop out in each treatment arm. Power is calculated for a two-sided t-test with significance level (alpha) 0.05 using PROC POWER in SAS 9.4.

While there will be multiple comparisons performed for this primary immunogenicity objective (for each of five parameters alone measured in CD4 and CD8 T cells plus any combinations measured), it is assumed that these analyses are not intended to be confirmatory and thus no adjustment for multiple comparisons is considered.

Table 10: Power Analysis for Primary Immunogenicity Endpoint

GMP_c/GMT_c {fold-change}	$\log_{10}(GMP_M)-\log_{10}(GMP_c)$ {log-difference}	Std Dev $\log_{10}$ (GMP)	Power (N = 60)	Power (N=54) 10% drop out
1.5	0.18	0.25	97	96
		0.50	49	45
		0.75	26	23
2	0.30	0.25	>99	>99
		0.50	90	87
		0.75	58	54
3	0.48	0.25	>99	>99
		0.50	>99	99
		0.75	93	90
4	0.60	0.25	>99	>99
		0.50	>99	>99
		0.75	99	98

11.4. Planned Interim Analyses

Interim analyses would only be used to terminate this trial in the event that unanticipated safety events deemed to be of sufficient concern require such action by the sponsor. These assessments will not be made on the basis of testing a formal statistical hypothesis; therefore, p-value adjustment will not be made to any analyses.

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11.4.1. Interim Safety Review

A DSMB will be convened by DMID to review study progress and participant, clinical, safety, reactogenicity, and immunogenicity data as described in section 9.6.2. Interim safety anlaysis will also be prepared as described in Section 11.4.2.

11.4.2. Interim Immunogenicity Review

Clinical, safety, and reactogenicity data through approximately Day 78 will represent the interim clinical database for this study. Once the last subject completes the visit that occurs approximately at Day 78, the interim clinical database will be cleaned, monitored and locked. An interim safety and immunogenicity report, with unblinded data presented aggregated by study arm, will include all available safety data, T cell responses after stimulation with M-001 peptides and with other Influenza A antigens at Baseline, Day 15, Day 36, Day 43 and Day 78, and M-001 ELISA responses at baseline, Day 22, Day 43, and Day 78. The interim report will be made available to the protocol team for planning subsequent trials and may be used for publication, but will not be used to make any decisions concerning the conduct of this trial.

As it is anticipated that subjects will remain in long term safety follow-up at the time of these analyses, the presentation of these data will not include any unblinded listings of individual subject data and aggregate summaries will be in a format that prevents inadvertent unblinding of the sponsor or clinical investigators to any individual subject's treatment assignment or immune response data. The interim analysis represents the final analysis of the included immunogenicity endpoints, and as such no p-value adjustment is required. This will also represent the final analysis of all reactogenicity data, unsolicited non-serious AEs through Day 43, and all clinical safety laboratory values. Available SAE data will be included in the interim report. Prior to the interim clinical database lock, a formal Statistical Analysis Plan that specifies all planned analyses will be finalized.

11.5. Final Analysis Plan

Once the last subject completes the final visit, the clinical database will be cleaned, monitored and locked. The CSR will be completed when all cellular and humoral immunogenicity data through day 28 following receipt of IIV3 are available.

11.5.1. Analysis Populations

The Safety Analysis population includes all subjects who received at least one dose of study vaccine.

The modified intent-to-treat (mITT) population includes all subjects who received at least one dose of study vaccine and contributed both at least one post-study vaccination venous blood samples for immunogenicity testing for which valid results were reported.

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The per protocol (PP) population includes all subjects in the ITT subset with the following exclusions:

- Data from all available visits for subjects found to be ineligible at baseline
- Data from all visits subsequent to major protocol deviations, such as:
    - Second or third study vaccination not received
    - Second or third study vaccination received out of window
    - Receipt of non-study licensed live vaccine within 30 days before or 21 days after each study vaccination
    - Receipt of non-study licensed inactivated vaccine within 14 days before or 21 days after each study vaccination
    - Receipt of non-study seasonal influenza vaccine (live or inactivated) within 30 days before or for the duration of the study
    - Receipt of immunosuppressive therapy (e.g., corticosteroids) within 30 days before or after each study vaccination
- Data from any visit that occurs out of window

In the case of mis-randomization, subjects will be analyzed according to the study product actually received for all analysis populations.

11.5.2. Safety Data

Summaries and analysis of safety data will be presented for the Safety Analysis Population.

Solicited AEs will be summarized by severity for each day after each study vaccination (Days 1-8 post each study vaccination) and as the maximum severity over all 8 days. Additionally, solicited AEs will be analyzed by taking the most severe response over the follow-up period, dichotomizing into a binary variable (none versus mild, moderate, or severe) and using standard techniques, such as exact confidence intervals, to summarize the proportion of subjects reporting each symptom, any injection site symptom, and any systemic symptom. Summaries of solicited AEs will be presented separately for each study vaccination as well as overall study vaccinations by treatment arm. The proportion of subjects reporting symptoms may be compared between treatment arms using Chi-square or Fisher's exact test, as appropriate. The proportion of subjects reporting solicited symptoms between the different study vaccinations (e.g., dose 1 vs. dose 2 or dose 3 vs dose 4) will be compared using McNemar's test.

Unsolicited AEs will be coded by Medical Dictionary for Regulatory Activities (MedDRA®) for preferred term and system organ class (SOC). The numbers of SAEs are expected to be small in this trial and will be reported by detailed listings showing the event description, MedDRA® preferred term and SOC, relevant dates (study vaccinations and AEs), severity, relatedness, and

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outcome for each event. Non-serious unsolicited AEs will be summarized as number and percentage of subjects reporting at least one event in each MedDRA® preferred term and SOC, cross tabulated by severity and relationship to study product. Additionally, the proportion of subjects and exact 95% confidence intervals of AEs in aggregate and by MedDRA® categories will be computed.

Clinical laboratory data will be summarized by severity for each visit and as the maximum over all post-study vaccination visits. Graphical presentations such as box plots will be used to illustrate the change from baseline.

11.5.3. Humoral Immunogenicity Data

Summaries and analysis of immunogenicity data will be presented for the ITT and PP populations; the PP population will be considered the primary analysis.

Immune responses in terms of strain-specific (IIV3 strains) HAI and Neut antibody titers will be summarized by treatment arm at each time point. Analyses at all time points will include proportion of subjects with titers ≥40 and geometric mean titers (GMTs). Additionally the proportion of subjects achieving seroconversion from Day 172 to Day 200 (defined as either a pre-vaccination titer <10 and a post-vaccination titer ≥40 or a pre-vaccination titer ≥10 and a minimum four-fold rise in post-vaccination antibody titer) will be presented. All summaries will include the corresponding 95% confidence intervals.

The proportion of subjects with seroconversion from Day 172 to 200 will be compared to evaluate the effect of M-001 priming using a two-sided Likelihood Ratio Test.

M-001 antibody response will be summarized by GMTs the proportion of subjects achieving seroconversion (defined as a minimum four-fold rise in post-vaccination ELISA antibody titer) at each time point, with corresponding 95% confidence intervals.

The correlation between HAI, Neut, and M-001 antibody titers will be evaluated. Plots such as reverse cumulative distributions or longitudinal presentations of GMTs will be presented for each assay.

11.5.4. Cell Mediated Immunity Data

Cellular immunogenicity assays will also be performed to assess if M-001 induces a cellular immune response, or primes for a cellular immune response to IIV3. T cell response will be measured as the percentage of T cell subsets expressing perforin, CD107a, IFN-γ, TNF-α, and IL2, alone or in combination in CD4 or CD8 T cells. For each measure at all time points, the GMP and corresponding 95% confidence interval will be presented. For the primary endpoint of T cell response against M-001 peptides 14 days post dose 2, a t-test will be used to compare GMPs between treatment arms.

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11.5.5. Missing Values and Outliers

All attempts will be made to collect all data per protocol. As missing data are expected to be minimal, no imputation will be performed for missing values. Any data point that appears to be erroneous or inexplicable based on clinical judgment will be investigated as a possible outlier. If data points are identified as outliers, sensitivity analyses will be performed to examine the impact of including or excluding the outliers. Any substantive differences in these analyses will be reported.

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12. SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Each participating VTEU site will maintain appropriate medical and research records for this trial, in compliance with ICH E6, Section 4.9, and regulatory and institutional requirements for the protection of confidentiality of subjects. Each participating VTEU site will permit authorized representatives of the DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical study records for the purposes of quality assurance reviews, audits, monitoring and evaluation of the study safety and progress. These representatives will be permitted access to all source data, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial. Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial.

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13. QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, the participating VTEU site is responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The site PI will provide direct access to all trial-related sites, source data/data collection forms, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The site PI will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

The SDCC will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the site(s) for clarification and resolution.

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14. ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1. Ethical Standard

The site PI will ensure that this trial is conducted in full conformity with principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR 46, 21 CFR 50 and 56, and ICH E6; 62 Federal Regulations 25691 (1997), if applicable. The site PI's Institution will hold a current Federal Wide Assurance (FWA) issued by the Office of Human Research Protection (OHRP) for federally funded research.

14.2. Institutional Review Board

Prior to enrollment of subjects into this trial, the approved protocol and informed consent form will be reviewed and approved by the appropriate IRB listed on its FWA.

The responsible official for the IRB will sign the IRB letter of approval of the protocol prior to the start of this trial and a copy will be provided to DMID. The IRB Federal Wide Assurance number will be provided to DMID.

Should amendments to the protocol be required, the amendments will be written by the sponsor and provided to the site PI for submission to the IRB.

14.3. Informed Consent Process

The site PI will choose subjects in accordance with the eligibility criteria detailed in Section 5. Before any study procedures are performed, subjects must sign an informed consent form that complies with the requirements of 21 CFR Part 50 and 45 CFR 46 and the local IRB.

Informed consent is a process that is initiated prior to an individual agreeing to participate in a trial and continuing throughout the individual's trial participation. Before any study procedures are performed, subjects will receive a comprehensive explanation of the proposed study procedures and study interventions/products, including the nature and risks of the trial, alternate therapies, any known AEs, the investigational status of the components, and the other elements that are part of obtaining proper informed consent. Subjects will also receive a detailed explanation of the proposed use and disclosure of their protected health information, including specifically their serum samples. Subjects will be allowed sufficient time to consider participation in the trial, after having the nature and risks of the trial explained to them, and have the opportunity to discuss the trial with their family, friends or legally authorized representative or think about it prior to agreeing to participate.

Informed consent forms describing in detail the study interventions/products, study procedures, risks and possible benefits are given to subjects. The informed consent form must not include any exculpatory statements. Informed consent forms will be IRB-approved and subjects will be

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asked to read and review the appropriate document. Upon reviewing the appropriate document, the site PI (or designee) will explain the research study to subjects and answer any questions that may arise. Subjects must sign the informed consent form, and written documentation of the informed consent process is required prior to starting any study procedures/interventions being done specifically for the trial, including administering study product.

DMID will provide the site PI, in writing, any new information that significantly impacts the subjects' risk of receiving the investigational product. This new information will be communicated by the site PI to subjects who consent to participate in the trial in accordance with IRB requirements. The informed consent document will be updated and subjects will be re-consented per IRB requirements, if necessary.

Study personnel may employ IRB-approved recruitment efforts prior to obtaining the subjects consent; however, before any study procedures are performed to determine protocol eligibility an informed consent form must be signed. Subjects will be given a copy of all informed consent forms that they sign.

By signing the informed consent form, subjects agree to complete all evaluations required by the trial, unless the subject withdraws voluntarily, or is withdrawn or terminated from the trial for any reason.

The rights and welfare of subjects will be protected by emphasizing to subjects that the quality of their medical care will not be adversely affected if they decline to participate in or withdraw from this trial.

14.4. Exclusion of Women, Minorities, and Children (Special Populations)

This trial will be inclusive of all adults who meet the Subject Inclusion/Exclusion Criteria, regardless of religion, sex, or ethnic background. Should the outcome of this trial be deemed acceptable, additional trials may be initiated in other populations.

It is unknown if the M-001 vaccine poses any risks to an unborn child. Female subjects of childbearing potential who are not surgically sterile via tubal sterilization, bilateral oophorectomy, or hysterectomy or who are not postmenopausal for ≥ 1 year must agree to practice highly effective contraception that may include, but is not limited to, abstinence from intercourse with a male partner, monogamous relationship with a vasectomized partner, male condoms with the use of applied spermicide, intrauterine devices, and licensed hormonal methods, with use of a highly effective method of contraception for a minimum of 30 days prior to study product exposure and agree to practice highly effective contraception for the duration of study product exposure, including 2 months (defined as 60 days) after the last study vaccination. A highly effective method of contraception is defined as one which results in a low failure rate (i.e., less than 1% per year) when used consistently and correctly. In addition to contraceptive use, all female subjects of childbearing potential will be required to have a negative serum or urine pregnancy test within 24 hours prior to receiving each dose of study vaccine. If a female

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subject becomes pregnant while participating in this study, we will ask her permission to follow-up with her about her health and the health of her baby through pregnancy outcome.

Children will not be included in this trial as presently there are no safety or efficacy data on this regimen in adults. Should the outcome of this trial be deemed acceptable, additional trials may be initiated in other populations.

14.5. Subject Confidentiality

Subjects will have code numbers and will not be identified by name. Subject confidentiality is strictly held in trust by the participating site PIs, their study personnel, the sponsor(s), and their agents. This confidentiality is extended to cover testing of biological samples, in addition to the clinical information relating to participating subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the trial or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All information provided by the Sponsor and all data and information generated by the participating VTEU site as part of the trial (other than a subject's medical records) will be kept confidential by the site PI and other study personnel to the extent permitted by law. This information and data will not be used by the site PI or other study personnel for any purpose other than conducting the trial. These restrictions do not apply to: (1) information which becomes publicly available through no fault of the site PI or other study personnel; (2) information which is necessary to disclose in confidence to an IRB solely for the evaluation of the trial (3) information which is necessary to disclose in order to provide appropriate medical care to a study subject; or (4) study results which may be published as described in Section 16. If a written contract for the conduct of the trial which includes confidentiality provisions inconsistent with this statement is executed, that contract's confidentiality provisions shall apply rather than this statement.

The study monitor, applicable regulatory authorities, such as the FDA, or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the site PI. This includes, but is not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this trial. The participating VTEU sites will permit access to such records.

To protect privacy, we have received a Certificate of Confidentiality. With this Certificate, the researchers cannot be forced to release information that may identify the research subject, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify the subject, except as explained below.

The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects, like this

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study, or for information that must be released in order to meet the requirements of the Federal Food and Drug Administration (FDA).

A Certificate of Confidentiality does not prevent the subject from voluntarily releasing information about themselves or their involvement in this research. If any person or agency obtains a written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

The Certificate of Confidentiality does not prevent the researchers from reporting without the subject's consent, information that would identify the subject as a participant in the research project regarding matters that must be legally reported including: child and elder abuse, sexual abuse, or wanting to harm themselves or others.

14.6. Study Discontinuation

If the trial is discontinued, subjects who sign the informed consent form, and are randomized and vaccinated will continue to be followed for safety assessments. No further study vaccinations will be administered.

14.7. Costs, Subject Compensation, and Research Related Injuries

There is no cost to subjects for taking part in this trial.

Subjects may be compensated for their participation in this trial. Compensation will be in accordance with the local IRB's policies and procedures, and subject to IRB approval.

If it is determined by the participating VTEU site and the site PI that an injury occurred to a subject as a direct result of the tests or treatments that are done for this trial, then referrals to appropriate health care facilities will be provided to the subject. Study personnel will try to reduce, control, and treat any complications from this trial. Immediate medical treatment may be provided by the participating VTEU site, such as giving emergency medications to stop immediate allergic reactions to the study vaccine. No financial compensation will be provided to the subject by the participating VTEU site for any injury suffered due to participation in this trial beyond that provided by clinical trial insurance and the PREP act.

14.8. Future Use of Stored Specimens

Residual samples/specimens are those that are left over after the study has been completed. Subjects will be asked for permission to keep any remaining (residual) samples (serum) derived from venous blood samples for possible use in future research studies, such as examining additional immunological assessments or testing for antibodies against other viruses or bacteria. Samples will be stored at a central clinical storage facility for future use and may be shared with investigators at the participating VTEU sites and with other investigators at other institutions once the clinical study report has been finalized. The samples will not be sold or used directly for production of any commercial product. No human genetic tests will be performed on samples.

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Each sample will be encoded (labeled) only with a barcode and a unique tracking number to protect subject's confidentiality.

There are no benefits to subjects in the collection, storage and subsequent research use of specimens. Reports about future research done with subject's samples will NOT be kept in their health records.

Subjects may be given the option to decide if they want their samples to be used for future research or have their samples destroyed at the end of this trial. The subject's decision can be changed at any time by notifying the study doctors or nurses in writing. However, if the subject originally consents to future use and subsequently changes his/her decision, any data from a previously collected sample may still be used for this research.

14.9. Disclosure of Individual Research Information

In this protocol, we intend to provide each participant with a lay summary of overall study results. In addition, participants may choose to receive their treatment assignment and will be informed about any risks and benefits of disclosure. Offering participants the option to decline and explaining the clinical significance of individual treatments is intended to minimize the negligible risks associated with disclosure. Interested subjects will be asked to provide contact information for receipt of the information. Results will be provided in an IRB-approved written document after the Clinical Study Report has been finalized.

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15. DATA HANDLING AND RECORD KEEPING

The site PI is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported.

Data collection forms will be derived from the eCRF and provided by the SDCC to record and maintain data for each subject enrolled in the study. All data collection forms should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making a change or correction, cross out the original entry with a single line and initial and date the change. Do not erase, overwrite, or use correction fluid or tape on the original.

Data reported in the eCRF derived from the data collection forms should be consistent with the data collection forms or the discrepancies should be explained.

The sponsor and/or its designee will provide guidance to site PIs and other study personnel on making corrections to the data collection forms and eCRF.

15.1. Data Management Responsibilities

All data collection forms and laboratory reports must be reviewed by the clinical team and data entry personnel, who will ensure that they are accurate and complete. AEs must be recorded on the appropriate data collection form, assessed for severity and relationship, and reviewed by the site PI or appropriate sub-I.

Data collection is the responsibility of the study personnel at the participating VTEU sites under the supervision of the respective site PI. During the study, the site PI must maintain complete and accurate documentation for the study.

The SDCC for this study will be responsible for data management, quality review, analysis, and reporting of the study data.

15.2. Data Capture Methods

Clinical (including, but not limited to, AE/SAEs, concomitant medications, medical history, physical assessments, and clinical laboratory values), reactogenicity, and immunogenicity data will be entered into a 21 CFR 11-compliant Internet Data Entry System provided by the SDCC.

The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the data collection forms completed by the study personnel.

15.3. Types of Data

Data for this study will include clinical, safety, and outcome measures (e.g., clinical laboratory values, reactogenicity, and immunogenicity data).

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15.4. Timing/Reports

An interim report will be generated as described in section 11.4.2. The clinical study report (CSR) will include clinical, safety, reactogenicity, and humoral and cellular immunogenicity data through the final visit. Once the last subject completes the final visit that occurs, the clinical database will be cleaned, monitored and locked. Unblinded analyses of safety, reactogenicity, and immunogenicity, including all primary and secondary endpoint data will be performed by the SDCC after the clinical database is locked. The CSR will be completed when all cellular and humoral immunogenicity data through day 28 following receipt of IIV3 are available.

After full analysis and final reporting is complete, and upon request and DMID approval, the SDCC will provide the participating VTEU sites with a summary of results by treatment arm and/or subject treatment assignments. In this regard, the participating VTEU sites requesting such information to share with study subjects must do so in compliance with their respective IRB guidelines.

15.5. Study Records Retention

Study records and reports, including, but not limited to, eCRFs, source documents, informed consent forms, and study drug disposition records, shall be maintained for 2 years after a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for the drug, until 2 years after the investigation is discontinued and FDA has been so notified. Informed consent forms for future use will be maintained as long as the sample exists.

The participating VTEU site must contact DMID for authorization prior to the destruction of any study records.

15.6. Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), or Manual of Procedures requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, section 5.1.1

5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site principal investigator and other study personnel to use continuous vigilance to identify and report deviations within five working days of identification

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of the protocol deviation, or within five working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID, via the SDCC's AdvantageEDC^{SM}.

All protocol deviations, as defined above, must be addressed in study subject DCFs. A completed copy of the DMID Protocol Deviation Form must be maintained in the Regulatory File as well as in the subject's chart. Protocol deviations must be sent to the local IRB per its guidelines. The site principal investigator and other study personnel are responsible for knowing and adhering to their IRB requirements.

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16. PUBLICATION POLICY

All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/) an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.

Refer to:

- NIH Public Access Policy, http://publicaccess.nih.gov/
- NIH Office of Extramural Research (OER) Grants and Funding, http://grants.nih.gov/grants/oer.htm

As of January 2018, all clinical trials supported by the NIH must be registered on ClinicalTrials.gov, no later than 21 days after the enrollment of the first subject. Results of all clinical trials supported by the NIH, generally, need to be submitted no later than 12 months following the primary completion date. A delay of up to 2 years is available for trials that meet certain criteria and have applied for certification of delayed posting.

As part of the result posting a copy of this protocol (and its amendments) and a copy of the Statistical Analysis Plan will be posted on ClincialTrials.gov.

For this trial the responsible party is DMID which will register the trial and post results.

The responsible party does not plan to request certification of delayed posting.

Refer to:

- Public Law 110-85, Section 801, Clinical Trial Databases
- 42CFR11
- NIH NOT-OD-16-149

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SUPPLEMENTS/APPENDICES

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APPENDIX A. SCHEDULE OF EVENTS

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Table 11: Protocol 14-0112, "A Phase II, Double Blind, Multicenter, Randomized, Placebo-controlled Trial to Assess the Safety, Reactogenicity and Immunogenicity of Two Doses of Multimeric-001 (M-001) Followed by Seasonal Trivalent Influenza Vaccine"

Study Visit (V)	01	02	03	04	05	06	07	08	09	10	11	12
Study Day	**1**	**9**	**15**	**22**	**30**	**36**	**43**	**78**	**172**	**186**	**200**	**200+**
Visit Windows		(+2)	(+2)	(+2)	(+2)	(+2)	(+3)	(+7)	142-202	(+3)	(+7)	(+14)
Sign Consent Form[1]	X											
Assess eligibility	X			X					X			
Review Medical History	X			X					X			
Concomitant meds	X	X	X	X	X	X	X	X[7]	X[7]	X[7]	X[7]	X[7]
Vital signs[2]	X	{X}	{X}	X	{X}	{X}	{X}	{X}	X	{X}	{X}	
Height, Weight	X											
Targeted Physical Exam	{X}	{X}	{X}	{X}	{X}	{X}	{X}	{X}	{X}	{X}	{X}	
Review contraception/ Counseling[3]	X	X	X	X	X	X	X	X	X	X		
Pregnancy Test[2,4]	X			X					X			
Blood - Safety Labs (10 mL)	X	X			X							
Blood – Antibody Assays (10-20 mL)	X			X			X	X	X*		X*	
Blood – PBMCs (40-60 mL)	X=		X			X	X=	X	X	X	X	
Randomization	X											
AE/SAE Assessment[2#]	X	X	X	X	X	X	X	X	X	X	X	X
Vaccination[4]	X[5]			X[5]					X			
Evaluate vaccination site	X	X		X	X							
Postvaccination procedures[6]	X			X								
Review Memory Aid Data		X			X							
Blood volume (mL)	80	10	40	10	10	40	70	50	60	40	60	

{ } required at this visit only if clinically indicated

[1]Consent process completed and form signed before any study-related procedures are conducted.

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[2]Vital signs include blood pressure, pulse, and oral temperature. On vaccination visits, these items should be completed prior to vaccination.

[3]Counseling on avoidance of pregnancy for women of childbearing potential.

[4]Urine or serum pregnancy test must be negative and be completed within 24 hours prior to vaccination for women of childbearing potential.

[5]All subjects will be observed for a minimum of 20 minutes following vaccination.

[6]Post-vaccination procedures will include documentation of any reactogenicity during the observation period and any AEs/SAEs post-vaccination, as well as provision of Memory Aid and instructions on completion.

[7] Concomitant medications (vaccines only) will be recorded on the appropriate data collection form after Visit 7 through the end of the study.

[+]Visit 12 will occur as a phone call only if visit 11 occurs prior to Study Day 200.

*20 mL of blood will be collected for antibody assays on these days; 10 mL will be collected on other days of collection for antibody assays

[=]60 mL of blood will be collected for PBMCs on these days; 40 mL will be collected on other days of PBMC collection

[#]AEs will be limited to SAEs after 21 days after M-001 study vaccinations.

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NIAID News and Science Writing Branch (NSWB)
Clearance Form

_X_ News Release ___Media Availability __Statement __NIH Record
__Other (specify): Blog post

Title: **NIAID-sponsored Trial of a Universal Influenza Vaccine Begins**
Audience: Media, interested public
Distribution Date/ Embargo Date: Friday, May 4
NSWB Contact/Writer: Elizabeth Deatrick
Comments: NIAID is sponsoring a Phase II trial of Biondvax's M-001 universal influenza vaccine candidate. The trial will test the vaccine when administered in two doses, in conjunction with a standard seasonal influenza vaccine. The trial will take place at three of NIAID's Vaccine and Treatment Evaluation Units (VTEUs).

DATE	NAME
_4/17____	Scientific Reviewers: Dr. Christopher Roberts, Viral Pathogens Clinical Research Program Officer, DMID Respiratory Diseases Branch
_4/17____	Scientific Reviewers: Dr. Wendy Keitel, Baylor College of Medicine (one of the VTEUs in this trial)
_4/17____	Scientific Reviewers: Tena Knudsen, Nurse Consultant, DMID Respiratory Diseases Branch
__4/17___	Branch/Program/Lab Chief: Dr. Diane Post, Acting Section Chief, Influenza, SARS, and Related Viral Respiratory Diseases Section, DMID Respiratory Diseases Branch
__4/17____	Division Director: Emily Erbelding, DMID director
_____	Budget Officer (as needed)
_____	Beat Supervisor:
_4/13____	Kathy Stover, NSWB Branch Chief
_4/13___	Courtney Billet, OCGR Director
__4/23_____	Laurie Doepel, Special Assistant to the Director
___4/24____	Carole Hudgings, Sr. Advisor to the Deputy Director
_4/23_____	Greg Folkers, NIAID Chief of Staff

Revised: 8/14/2015

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_____	Hugh Auchincloss, NIAID Deputy Director
_____	Anthony S. Fauci, NIAID Director
4/17	Other Reviewers: Josh Phillipson and others at BiondVax
4/24	Hilary Marston
4.24	Cat Paules
4/24	Bob Eisinger

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Mon, 30 Apr 2018 18:11:48 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Folkers, Greg (NIH/NIAID) [E];Vance, Meaghan (NIH/NIAID) [C];Billet, Courtney (NIH/NIAID) [E]
Subject: FW: question from Mother Jones - univ flu

Pls advise.

Patricia L. Conrad
Special Assistant to the Director

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The information in this e-mail and any of its attachments is confidential and may contain sensitive information. It should not be used by anyone who is not the original intended recipient. If you have received this e-mail in error please inform the sender and delete it from your mailbox or any other storage devices. National Institute of Allergy and Infectious Diseases (NIAID) shall not accept liability for any statement made that are sender's own and not expressly made on behalf of the NIAID by one of its representatives.

From: Vance, Meaghan (NIH/NIAID) [C]
Sent: Monday, April 30, 2018 2:09 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: FW: question from Mother Jones

From: Kiera Butler [mailto:kbutler@motherjones.com]
Sent: Monday, April 30, 2018 1:50 PM
To: Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Subject: question from Mother Jones

Hi Meaghan,

Kiera at Mother Jones here—I'm writing a quick post on Trump's comments on universal flu vaccine with Bill Gates. Here's the STAT piece about it. Any chance Anthony Fauci might be willing to offer a quick comment today? He was really helpful when I was writing on universal flu vaccine research a few months ago!

Thanks!

Kiera

From: "Vance, Meaghan (NIH/NIAID) [C]" (b)(6)
Date: Thursday, March 22, 2018 at 3:44 PM
To: Kiera Butler <kbutler@motherjones.com>
Subject: RE: interview with Dr. Fauci

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Would you have time right now? Dr. Fauci has a few free minutes to talk....

From: Kiera Butler [mailto:kbutler@motherjones.com]
Sent: Thursday, March 22, 2018 3:43 PM
To: Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Subject: Re: interview with Dr. Fauci

Hi Meaghan,

Thanks for getting back to me—yes, 2 PM tomorrow would work.

Best,

Kiera

From: "Vance, Meaghan (NIH/NIAID) [C]" (b)(6)
Date: Thursday, March 22, 2018 at 3:37 PM
To: Kiera Butler <kbutler@motherjones.com>
Subject: interview with Dr. Fauci

Kiera:

Dr. Fauci would be happy to speak with you about the new CDC director Robert Redfield and his background in HIV research. He has the following availability tomorrow:

8:30am, 9:30am or between 2-3pm

Will any of these times work?

Thank you,
Meaghan

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From: Collins, Francis (NIH/OD) [E]
Sent: Mon, 30 Apr 2018 14:33:10 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Re: Gates and POTUS

I'm at the Milken thing in LA, will try to call later..

Sent from my iPhone

On Apr 30, 2018, at 7:06 AM, Fauci, Anthony (NIH/NIAID) [E] (b)(6) wrote:

Francis:
We should really talk by phone if possible. This is really disturbing. You and I have been talking about a universal influenza vaccine for a few years now https://www.ncbi.nlm.nih.gov/pubmed/21135852 . In June, 2017 we had our national and international Workshop on Universal Influenza vaccine that led to a brief summary of the workshop https://www.ncbi.nlm.nih.gov/pubmed/29045889 followed by our strategic plan and research agenda, which we also published https://www.ncbi.nlm.nih.gov/pubmed/29506129. Much of what Bill discussed came out of our plan and his visit to the VRC last year. In addition, we have been the lead in discussing the need for a universal influenza vaccine https://www.ncbi.nlm.nih.gov/pubmed/29185857 . In Bill's Shattuck Lecture on April 27, 2018, he mentioned "We are also supporting efforts by others including the National Institute of Allergy and Infectious Diseases, whose candidate vaccine is expected to advance to human safety trials in about a year". He is pledging a one time infusion of $12 million and we spend $104 million in our base per year with more to come.
Happy to chat.
Best,
Tony

From: Collins, Francis (NIH/OD) [E]
Sent: Monday, April 30, 2018 9:27 AM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Tabak, Lawrence (NIH/OD) [E] (b)(6) Wolinetz, Carrie (NIH/OD) [E] (b)(6)
Subject: Gates and POTUS

Hi Tony,

See https://www.statnews.com/2018/04/30/bill-gates-vaccine-trump-meeting/

Interesting report of a 40 minute meeting that Gates had with POTUS, where universal flu vaccine came up. POTUS called Gottlieb, who didn't have a lot to say. Gates doesn't seem to know there's a strategic plan. Should we be following up?

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Mon, 30 Apr 2018 13:42:16 +0000
To: Conrad, Patricia (NIH/NIAID) [E]
Subject: FW: Following up on global HIV response discussion
Attachments: PreConference agenda draft MARCH 30.docx, Fast Track report draft outline, Post Noordwijk Project Description - Revision July 16.docx

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From: David Barr (b)(6)
Sent: Monday, April 30, 2018 9:03 AM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Peter Staley (b)(6) James Krellenstein (b)(6)
Subject: Following up on global HIV response discussion

Tony -

I'm following up on our discussion at dinner last week regarding the work I am doing with the Joep Lange Institute to identify and address challenges to sustaining success in the global HIV response. I have attached three documents. One is an outline of the final report from the project, which we are now drafting for release in July. The second is the agenda for the full day preconference session that we will hold at AIDS2018 in Amsterdam on Sunday, July 22nd. The third is a longer document that describes the focus of our work in greater detail.

The project has focused on the challenges posed in meeting and sustaining current UNAIDS and PEPFAR targets, based on the data that has been collected over the past few years since Fast Track was initiated. Our overarching areas for review have been:

- How to prioritize HIV spending if resources are insufficient to cover everything recommended within the Fast Track initiative
- How to ensure the sustained quality of care in treatment delivery and the potential dangers of treatment initiation if quality cannot be assured
- The need for revitalization of HIV primary prevention given that treatment alone will not lead to the level of incidence reduction necessary for epidemic control
- The use of innovative financing mechanisms for the HIV response and the integration of HIV within Universal Health Coverage approaches

In addressing these issues, I have organized seven meetings over the past year, each bringing together a diverse group of public health officials, researchers, economists, activists, and health care providers. The final report will reflect the results of these discussions and provide recommendations for addressing these challenges.

Your thoughts on any of this is, of course, very welcome. Perhaps once I have the recommendations finalized we can discuss further to see how you might be able to participate in follow up steps.

Thanks again for dinner. Such a nice evening.

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David

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DRAFT

Efforts to Scale-up, Strengthen, and Sustain HIV Responses:

- **Protect the quality of HIV treatment delivery**
- **Scale Up community-based service delivery and advocacy**
- **Reinvigorate primary prevention**
- **Develop sustainable and equitable funding approaches**
- **A commitment to human rights protection**

AIDS 2018 Pre-conference
July 22, 9:00 AM – 5:00 PM

9:00 – 9:05:	Welcome and Introduction Michiel Heidenrijk, Joep Lange Institute
9:05 – 9:15	The need for new strategies in the global HIV response: Project overview: review of the overall project, why it was developed and how it was implemented - David Barr, Joep Lange Institute
9:15 – 10:00	Efforts to Scale up, Strengthen, and Sustain HIV Responses: Findings and recommendations: (Peter van Rooijen – moderator) • Putting people living with HIV first: Protecting the quality of HIV treatment scale up – Lillian Mworeko, International Community of Women Living with HIV (ICW) East Africa - Uganda • Treatment alone will not end AIDS: A commitment to scale up of HIV primary prevention - Nduku Kilonzo, National AIDS Control Council – Kenya • The future of funding the HIV response – Mark Dybul, Georgetown University Center for Global Health and Policy – United States
10:00 – 10:15	Coffee break
10:15 – 11:30	Panel: The Global Response (Wafaa El Sadr – moderator) • Debbie Birx – PEPFAR • Tedros Adhanom Ghebreyesus – WHO (TBC) • Michel Sidibe – UNAIDS • Peter Sands – The Global Fund (TBC) • Maureen Murenga, Kenya (TBC) • Rico Gustav, ICSS, the Netherlands (TBC)

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11:30 – 12:30	Setting priorities in an era of constrained funding: Lessons from the Front (Peter Reiss (TBC) – moderator) • Fast Track city 1 - Mayor Vitali Klitschko, Kiev, Ukraine (TBC) • Fast Track city 2 – Mayor Mr Baibek Bauyrjan, Almaty, Kazakhstan (TBC) • Fast Track city 3 – Bangkok (TBC)
12:30 – 13:30	Lunch
13:30 – 14:30	A lifetime of treatment - challenges to sustainable HIV treatment success: (Ale Tressaro – moderator) • Wame Mosime, ITPC - Botswana • Francois Venter, University of Witswatersrand - South Africa • Daola Diouf, Sante Sana – Senegal • Tsitsi Apollo, Ministry of Health and Child Care – Zimbabwe • Meg Doherty – WHO (TBC)
14:30 – 15:30	Controlling the epidemic: A Renewed Commitment to Primary Prevention (Geoff Garnett – Moderator) (TBC) • A Call to Action: Current status of HIV prevention efforts among key populations, young adults and adolescents – Linda Gail Bekker, Bishop Desmond Tutu Center - South Africa (TBC) • A proposed model of differentiated service delivery for HIV prevention - Wanjiro Mukoma – LVCT - Kenya • Measuring HIV incidence among key populations, young adults and adolescents - Jessica Justman, ICAP – United States • A commitment to harm reduction: Anya Sarang, Andrei Rylkov Foundation – Russia (TBC)
15:30 – 15:45	Break
15:45 – 16:45	Innovative financing: what are countries doing now? (John Simon – moderator) • Implementing UHC: Sylvester Ziniel, Ministry of Health – Ghana • Implementing UHC: Youssoupha Ndiaye, Ministry of Health and Social Action –Senegal (TBC) • Implementing UHC: Jonas Bargas, APCASO - Thailand (TBC) • Funding community systems: Caroline Gomes – Dominican Republic

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16:45 – 17:00 Closure and Farewell (Michiel Heidenrijk – moderator)

- Project Chairs (Dybul, Kilonzo, Mworeko)

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Ensuring Efforts to Scale up, Strengthen and Sustain HIV Responses
A new global initiative aimed at maximizing impact and efficiencies through smarter, targeted approaches

1. Introduction and Overview

Broadly speaking, the global scale-up of HIV treatment has met with remarkable success, bringing life-prolonging antiretroviral therapy (ART) and essential supportive services to millions of persons living with HIV. However, some countries are struggling to reach large shares of their overall population with HIV information, prevention, care and treatment, while other countries have epidemics characterized by widespread failure or inability to provide certain subpopulations—such as gay and bisexual men, sex workers, people who inject drugs and transgender women (among other key populations), adolescents and young adults—with access to essential HIV services. These disparities will hinder efforts to achieve the level of prevention and treatment coverage required to control the epidemic. In addition, external funding for the HIV response has contracted, and is not expected to be adequately replaced with domestic resources or otherwise rise to the amounts specified as necessary in the UNAIDS Fast-Track initiative.

Given these challenges, new and innovative approaches for HIV service delivery, resource mobilization and investment strategies are urgently needed to maintain and build upon the successes to date. Strategies must be developed to focus more effectively on reducing the number of new infections. Treatment and prevention services should be better targeted to the individuals and settings where they are most required, and such services must be shaped to better meet the needs of those to be served. Potential opportunities exist to reduce costs, improve service delivery, and develop sustainable funding mechanisms. Exploring these opportunities is the crucial next phase of the global HIV response.

'Ensuring Efforts to Scale up, Strengthen and Sustain HIV Responses' is a new initiative that will seek to identify realistic, evidence-based options and solutions for this next phase that are relevant for global, regional and local stakeholders. Hosted by the Joep Lange Institute[1], its objectives are to articulate an approach for the HIV response that takes sharp aim at reducing the number of new HIV infections, streamlining and improving service delivery, efficiently targeting the use of resources, and building new and more sustainable approaches for funding. The initiative also will provide detailed information and guidance for advocates, policy makers and others by examining the potential impact of reduced funding for the HIV response on scale-up strategies and through identification of key areas in which additional implementation research and/or advocacy is needed for continued scale-up of effective services.

Three chairpersons will oversee the project:

- Dr. Kesetebirhan (Kesete) Admasu, Chief Executive Officer of Roll Back Malaria and the former Ethiopian Minister of Health
- Dr. Mark Dybul, Director of the Center for Global Health and Quality at Georgetown University School of Public Health and former Director of the Global Fund to Fight AIDS, TB and Malaria

[1] Based in Amsterdam, the Joep Lange Institute is a not-for-profit organization that describes its work at combining "science, activism, and pragmatism to reach our goal: making health markets work for the poor where the system fails the people." More information is available at www.joeplangeinstitute.org.

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- Cindy Kelemi, Executive Director of the Botswana Network on Ethics, Law and HIV and AIDS (BONELA)

A Planning Committee met in April 2017 in Noordwijk, the Netherlands to develop a guiding framework and approach for the initiative. Its members are from a wide range of constituency groups including health ministries, public and private donors, advocates and people living with HIV, health care providers, clinical and behavioural researchers, and economists.

The project's primary work will take place through three workstreams, each comprising participants from diverse geographical, professional and sector contexts. The three workstreams' topics include (1) the potential impact of reduced funding on scale-up strategies; (2) optimizing incidence reduction and treatment efforts, including through differentiated service delivery (DSD) approaches; and (3) developing and proposing financing strategies for the short and longer term. (Each workstream is discussed in greater detail in Section 3 below.)

The main output will be a consolidated report based on the deliberations and outcomes from each large workstream meeting and other relevant workstream-specific work. The report's launch and the roll out of associated advocacy and communications strategies to disseminate resulting ideas and information will take place at the July 2018 International AIDS Conference in Amsterdam.

2. Overarching and Cross-cutting Issues

Preliminary discussions with initiative participants, including at the April 2017 Planning Meeting, highlighted several overarching and cross-cutting issues that underscore the need for and scope of the project. Those issues and themes include the following, each of which will be analysed in detail throughout the process and considered across all three workstreams:

- Reducing ***HIV incidence*** should be a central focus of HIV programmes. To this end, access to incidence data among specific populations and subnational geographic areas is a high priority: an effective HIV response must understand where and how people are getting infected.

- The 90-90-90 targets are helpful motivators and milestones for HIV scale-up, but may be insufficient to end the epidemic in many countries, where the people in "***the last 10 percent***" may be drivers of onward transmission and propagation of micro-epidemics. To end the HIV epidemic, it is necessary to know who is missing, why they are being missed, and what programmes and services will best engage them and meet their needs.

- Achieving epidemic control relies on emphasizing the further development of HIV programmes that are better attuned to the contexts and priorities of the people they are serving. Such ***differentiated service delivery (DSD)*** models aim to better meet client needs and, at the same time, reduce the burden on health care providers and health facilities.

- It is vital to refine and nuance our understanding ***of socio-structural factors*** that conspire to create individual vulnerabilities. Social scientists, including and not limited to, anthropologists, psychologists, and sociologists, are needed to help refocus all HIV responses on people, not on approaches or interventions.

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- External financing to support the HIV response is unlikely to increase and may even continue to decline in the coming years. Some middle-income countries (MICs) might have the ability to support some elements of their HIV responses through domestic resources, but this is not a realistic expectation for low-income countries (LICs). While advocacy for continued and increased domestic and international resources for the HIV response must continue, ***alternative investment strategies*** over the longer term are needed.

- ***Insufficient funding levels*** will require thoughtful prioritization of treatment and prevention interventions, but may raise tensions about competing resource needs for these programmes. Guidance is needed to assist countries as they struggle to allocate resources to address a full range of HIV health needs. Major risks from gaps in supply and services include increased drug resistance, harmful and stigmatizing competition for access, and expansion of treatment access in ways that are not safe and ethical.

- ***Adolescents and young people*** are less likely to be successfully engaged in HIV care and prevention. Recent findings from rigorous surveys in Malawi, Zambia and Zimbabwe indicate that just 40 percent of 15–19 year olds knew their HIV-positive status, compared with around 80 percent of those older than 25[2]. Many young people report a lack of knowledge about HIV transmission and how to avoid it. Far too little is known about these young people, who are clearly not being reached by prevention and treatment services.

- Significant opportunities exist to improve ***linkage and retention in care***. Expanding and capitalizing on these opportunities could also lead to reduced costs through the simplification of methods for drug delivery, including fewer required clinic visits. Re-evaluation of the type and frequency of diagnostic and monitoring tests needed can make it easier for people living with HIV to engage in care, reduce burdens on health care workers, and potentially lower costs.

- More attention and resources should be directed toward ***HIV prevention***. A more sophisticated understanding is needed about what works and for whom. Prevention efforts in general are most effective when combination approaches (e.g., testing + condoms + lube + PrEP + harm reduction programmes) are employed and integrated with other health/sexual health services (e.g., social support + mental health counselling + substance abuse treatment services).

- Stigma, discrimination, and violence harm health at both the individual and population levels. ***Human rights protection*** is a central, cross-cutting issue in all aspects of the HIV response. Public health goals can only be achieved through a better understanding of and commitment to address the individual, social, structural and human rights factors that drive vulnerability to HIV infection and limit access to prevention and treatment.

- HIV can no longer be dealt with in isolation as a siloed health concern. Greater consideration must be given to a wide range of ***other areas and issues where HIV-specific progress can be made***, such as the provision of sexual and reproductive health services; education; and social programmes, including mental health and substance abuse

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[2] These surveys were part of the ongoing PHIA Project undertaken by ICAP, based at Columbia University in New York City. PHIA stands for 'population HIV impact assessment'. More information is available at http://phia.icap.columbia.edu/.

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services. Concomitant consideration should be given as well to the various government agencies and other stakeholders that provide and fund such services.

3. Project Workstreams

Summaries are provided below of the three workstreams that will collaboratively contribute to the comprehensive final report.

Workstream One: The Potential Impact of Reduced Funding for HIV on Scale Up Strategies

Current situation and rationale for prioritization:
Global donor HIV investment levels fell by approximately $1 billion in 2015. Most notably, donors are sharply reducing funding for middle-income countries, where the majority of people living with HIV now reside—a majority that is expected to increase as more countries are reclassified as middle income. HIV responses in these countries will increasingly have to rely on substantial increases in domestic investment. However, many countries will be unable to invest at these levels and some may not be willing to make such investments given the high numbers of populations with HIV and at risk for HIV, including marginalized key populations that live there. In lower-income countries, there are questions as to whether estimated resource allocations will be sufficient to meet the needs of the proposed scale up under Fast-Track. If the required resource increases do not materialize, then alternative funding estimates and plans are needed to inform the prioritization of resources. Currently, there is no "Plan B" and this is a potentially risky situation.

Priority questions to consider:

- How do weak links in drug supply chains, human resource capacity, and growing rates of drug resistance affect the rate and quality of treatment scale-up?
- Where are the opportunities to reduce costs? Can cost reduction alone make up for reduced funding levels?
- How are countries prioritizing funding allocations now? What is driving those priorities? Could re-prioritization improve outcomes and save resources?
- What is the value of an HIV-specific approach for the near and long-term? How can, or should, the structural factors that drive vulnerability to HIV infection and illness, particularly among key populations and young people, be better addressed?
- What kinds of ethical and strategic guidance for programme prioritization are needed to support countries facing low or decreasing HIV funding levels?

Workstream Two: How can prevention and treatment efforts be optimized to reduce the number of new HIV infections and move towards ending HIV epidemics in every location and population? How can differentiated service delivery (DSD) be designed and implemented to maintain and accelerate treatment and prevention?

Current situation and rationale for prioritization:
As recommended at a March 2017 UNAIDS-sponsored meeting on HIV scale-up implementation and progress[3], the next phase of the global HIV response requires specific focus and greater emphasis on reducing infection rates, reaching those most vulnerable and at

[3] *Close the tap of new HIV infections: Revitalize HIV combination prevention in eastern and southern Africa region: Expert meeting to fast-track HIV prevention implementation in 15 fast-track countries.* The meeting was held in Victoria Falls, Zimbabwe.

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risk, and addressing the structural factors that increase vulnerability and hinder access to prevention and treatment. All of these imperatives underscore the priority value of tailoring service delivery to better respond to patient needs and preferences, an intensive focus on high-incidence localities and populations, streamlining services in ways that reduce the burden on health systems and reduce costs, and expanding the role of community systems in creating demand for and use of health and social services. Most countries will face a core challenge in developing this differentiated approach within a public health structure that can provide services at scale in under-resourced health systems.

Priority questions to consider:

- What data and information are needed to better understand where HIV micro-epidemics are located, whom they affect, and why they persist?
- Is the knowledge base regarding how to implement prevention, treatment and care programmes within micro-epidemic and key populations sufficient to affect infection rates and disease progression? Are current cost estimates accurate for these services?
- What is the package of primary prevention interventions that can be prioritized to address the needs of key and high incidence populations?
- How might rising rates of drug resistance affect policies regarding treatment initiation?
- Can an increase in focus on incidence reduction in micro-epidemics be implemented while maintaining overall treatment scale up and retention services?
- How can HIV be better incorporated into broader efforts to address social determinants of improved health, economic equity, and quality of life?
- What financial, advocacy and research investments are needed to influence the political will to achieve a strengthened focus on highly vulnerable and marginalized populations in the global HIV response and in all local/national responses? What mechanisms are best suited to support this work?
- What might a highly effective and implementable DSD model look like throughout each stage of the HIV prevention and treatment cascades? What are the metrics and support systems that can measure the effectiveness of DSD approaches and help ensure that all health care personnel are providing what is expected?
- What are the costs for development, scale up and implementation of DSD approaches? Does/can DSD lower overall costs for health care delivery?
- Can adoption and scale up of DSD interventions be prioritized based on their effectiveness, their ability to reduce the burden on health care systems, cost, timeline and complexity of implementation, and potential degree of impact?
- What is the role of community systems in DSD and what is needed to support this infrastructure to fulfil its role?
- What changes are needed in legislation and regulation, and in critical logistical areas such supply chain management, for effective DSD implementation?
- What is the role of government sectors beyond health in providing information and services to and for people living with and at risk for HIV? What are the barriers to a multi-sector approach to client-centred services?

1. **Workstream Three: What are short-, medium-, and long-term financing strategies for optimal delivery of HIV treatment and prevention?**

Current situation and rationale for prioritization:

Resource levels for the HIV response have not reached the amounts required as estimated by the UNAIDS Fast-Track initiative. Short-term strategies are needed to determine how best to use the limited resources currently available for HIV treatment and prevention. Longer-term

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strategies require the development of innovative financing mechanisms that can support and continue the scale-up of effective HIV treatment and prevention programmes in the context of a commitment to universal health care and sustainable development. The usefulness of such strategies relies on more accurate and comprehensive country-specific data on costs for services, to better understand cost drivers and to inform planning for resource mobilization and budgeting processes. Also, more information is needed about the true potential for increasing domestic resources in general, and specifically by country.

Priority questions to consider:

Short term

- How can resource-limited countries best evaluate the clinical, epidemiological, and budgetary consequences of alternative HIV programme scale-back strategies?
- How are resource-limited countries currently prioritizing allocations for HIV treatment, prevention and care?
- Are current resource needs estimates reliable and accurate? Which areas for saving would produce the least harm to both individuals and the public health?

Medium term

- What are the arguments in support of continued HIV-specific funding within the overarching push for universal health coverage and sustainable development?
- What arguments and data are needed to convince ministers of finance and other ministry decision makers to invest in innovative funding opportunities?
- What are costs for services provided by community members and non-professionals, and are these services sustainable over the long term?
- What are the overall financial consequences of increasing HIV drug resistance to standard 1st line regimens (both adult and paediatric), as implied by switching to costlier 2nd line regimens? What are the options for 2nd line? How to address 3rd line options in resource-constrained settings?

Long term

- What are the advantages and limitations of innovative financing mechanisms such as insurance schemes, remittances, blended financing, buy-down of loans, endowments, personal saving and remittance prepayment mechanisms (electronic and otherwise), health bonds and performance-based funding to support HIV responses?
- What are the benefits that scaling up of (public) insurance schemes can bring to the HIV response?
- How can innovative financing approaches be prioritized for implementation? What is the role of advocates and other stakeholders in advancing uptake of these approaches?

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From: Paules, Catharine (NIH/NIAID) [E]
Sent: Mon, 30 Apr 2018 13:20:02 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: FW: Request for Dr. Fauci from AAI--followup

FYI regarding the commentary for Journal of immunology. Important points highlighted. Author list is detailed below.

From: Fox, Ellen (b)(6)
Sent: Friday, April 27, 2018 5:07 PM
To: Paules, Catharine (NIH/NIAID) [E] (b)(6)
Cc: Folkers, Greg (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6) Hogan, Michele (b)(6) Oltz, Gene (b)(6) Reitzel, Todd (b)(6)
Subject: RE: Request for Dr. Fauci from AAI--followup

Dear Dr. Paules:

Thank you for your message. We are honored to have Dr. Fauci contribute a commentary to "Immunity to Influenza: A Moving Target", a topical review issue scheduled for January 15, 2019 in *The Journal of Immunology*.

1. We have confirmed the following authors and topics for this special issue:
   James Crowe, Vanderbilt Vaccine Center antibody repertoire to influenza infection
   Linda Wakim, The University of Melbourne, Doherty Institute memory T cell dynamics in the lung
   Nicole Baumgarth, University of California, Davis B cell responses to influenza
   Matthew Miller, McMaster University original antigenic sin
   Jacob Kohlmeier, Emory University modeling flu responses
   Katherine Kedzierska, The University of Melbourne, Doherty Institute human Tfh responses to influenza
   Kanta Subbarao, The University of Melbourne, Doherty Institute avian influenza
   Jacco Boon, Washington University School of Medicine in St. Louis flu structure
   Stacy Schultz-Cherry, St. Jude Children's Research Hospital development of a universal flu vaccine
   TBD innate immunity to influenza

Dr. Fauci inquired earlier if he could have a co-author; he may, and we do not have any opposition to him recruiting any of the above authors on his commentary.

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2. We would appreciate receiving the commentary by **September 1, 2018.** When you are ready to submit the commentary, you may send it directly to me (b)(6)

3. We do not have any specific length or reference requirements for this commentary, however, as is standard, previous commentaries have been approximately 2 – 3 pages. If you wish to estimate manuscript length, one printed page in *The Journal of Immunology* is approximately 8,000 characters including spaces. We anticipate that the reviews published in this issue will be approximately 48,000 characters (5 – 6 pages), including spaces and excluding references.

4. Just to reiterate, there are no charges or fees for this commentary.

Please do not hesitate to contact me with any other questions.

Sincerely,

Ellen C. Fox, Ph.D.
Science Associate I
The Journal of Immunology and *ImmunoHorizons*
The American Association of Immunologists
1451 Rockville Pike, Suite 650
Rockville, MD 20852
Ph. (b)(6)
FAX 301-634-7829
(b)(6)

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From: Paules, Catharine (NIH/NIAID) [E] (b)(6)
Sent: Friday, April 27, 2018 2:03 PM
To: Fox, Ellen
Cc: Folkers, Greg (NIH/NIAID) [E]; Conrad, Patricia (NIH/NIAID) [E]; Vance, Meaghan (NIH/NIAID) [C];

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Hogan, Michele
Subject: Request for Dr. Fauci from AAI--followup

Hello Dr. Fox,
Dr. Fauci asked me to follow up with you regarding the invited commentary for the 2019 topical review-"immunity to Influenza: A Moving Target. Do you have any further information on the proposed topics for the issue and who the other authors may be? Additionally, should we still plan on a submission date of September 1st and do you have any length or reference requirements you would like us to follow. Thank you very much,
Cat

Catharine Irene Paules, M.D.
Medical Officer
Immediate Office of the Director
National Institute of Allergy and Infectious Diseases
National Institutes of Health
(b)(6)

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Wednesday, March 28, 2018 4:26 PM
To: 'Hogan, Michele' (b)(6)
Cc: Gene Oltz (b)(6) (b)(6) Wayne M. Yokoyama (b)(6) JoAnne Flynn (b)(6) (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: RE: Request from AAI

Michele:
Thanks for the note. I already spoke to Ellen Fox and confirmed that I would do this.
Best regards,
Tony

From: Hogan, Michele (b)(6)
Sent: Tuesday, March 27, 2018 6:25 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Gene Oltz (b)(6) (b)(6) Wayne M. Yokoyama (b)(6) JoAnne Flynn (b)(6) (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: Request from AAI

Dear Tony:

Attached please find our invitation regarding a special issue of *The Journal of Immunology*.

With best regards,
Michele

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M. Michele Hogan, Ph.D.
Executive Director
The American Association of Immunologists
1451 Rockville Pike, Suite 650
Rockville, MD 20852
(b)(6)
www.aai.org

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From: Billet, Courtney (NIH/NIAID) [E]
Sent: Mon, 30 Apr 2018 13:19:45 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: ASF --

Another one

http://thehill.com/policy/healthcare/385459-bill-gates-trump-super-interested-in-universal-flu-vaccine

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From: Grady, Christine (NIH/CC/BEP) [E]
Sent: Sun, 29 Apr 2018 23:47:17 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: RV490 consent form 180316 Main Study clean.docx
Attachments: RV490 consent form 180316 Main Study clean.docx

Print but don't bother with first coupleof pages

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From: Harris, Kara (NIH/NIAID) [E]
Sent: Sun, 29 Apr 2018 21:42:40 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Conrad, Patricia (NIH/NIAID) [E]
Subject: Re: Input: 372243 Natsios (Snowcroft Invitation for Dr. Collins)

Dr. Fauci,

Thank you for your input.

Kara

On Apr 28, 2018, at 2:46 PM, Fauci, Anthony (NIH/NIAID) [E] (b)(6) wrote:

I did something similar for these people last year and would probably do it again in the future since I do biodefense and pandemic preparedness all the time. It is at the National Press Cloud and so on logistic grounds alone, the audience is not that large. As for Dr. Collins, with all the other things that are going on for him, I am not so sure it is worth his time to step into biodefense and pandemic preparedness since they usually leave a lot of time for questions and he would have to bone up on specifics.

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Friday, April 27, 2018 9:37 AM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: FW: Input: 372243 Natsios (Snowcroft Invitation for Dr. Collins)

What say you.....

Patricia L. Conrad
Special Assistant to the Director

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From: Harris, Kara (NIH/NIAID) [E]
Sent: Friday, April 27, 2018 8:47 AM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Billet, Courtney (NIH/NIAID) [E] (b)(6) Haskins, Melinda (NIH/NIAID) [E] (b)(6)
Subject: Input: 372243 Natsios (Snowcroft Invitation for Dr. Collins)

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Hi, Patty –

Dr. Collins has received the attached invitation to be the keynote speaker at the 2nd Annual Pandemic and Biosecurity Policy conference sponsored by the Snowcroft Institute of International Affairs at the Bush School at Texas A&M University. The conference will focus on the coming threats from pandemic disease and international health security. It is being held on the morning of May 26, 2018, at the National Press Club in Washington, DC.

Building 1 is requesting Dr. Fauci's input on whether he should accept or decline this invitation. They would like our response by 2:00 p.m. on Monday.

Thanks for your help!
Kara

Kara M. Harris, MPH
Section Chief for Controlled Correspondence and Public Inquiries
Office of Communications and Government Relations
National Institute of Allergy and Infectious Diseases
National Institutes of Health
5601 Fishers Lane, Room 6F30
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From: (b)(6)
Sent: Sun, 29 Apr 2018 00:17:46 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: RE: Question
Attachments: 01 WEVEE VLP paper_v22.docx, 01 WEVEE VLP_Figures v21 (3).pdf, Kamp et al Lyme Apr 23 2018 Nature.docx

Hi Tony,

(b)(4)

Many thanks again for your time, effort and consideration in supporting my NAS nomination. I really appreciate it!

Best
Gary

From: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Sent: Saturday, April 28, 2018 4:01 PM
To: Nabel, Gary /US
Subject: [EXTERNAL] Question

Gary:
I am going to be presenting your nomination for NAS at the Annual Meeting on Monday. In the 50 WORD statement that we put together, we make the statement: "Nabel discovered the first effective vaccine candidates for Ebola, Chikungunya and encephalitis viruses through his approach to rational design using molecular virology, structure and immunology." What specific "encephalitis" vaccine candidates are we referring to? I cannot find this in the 12 references that we provided.
Thanks,
Tony

Anthony S. Fauci, MD
Director
National Institute of Allergy and Infectious Diseases
Building 31, Room 7A-03

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31 Center Drive, MSC 2520
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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Sat, 28 Apr 2018 19:12:43 +0000
To: Marston, Hilary (NIH/NIAID) [E]
Cc: Folkers, Greg (NIH/NIAID) [E];Vance, Meaghan (NIH/NIAID) [C];Conrad, Patricia (NIH/NIAID) [E];Doepel, Laurie (NIH/NIAID) [E];Eisinger, Robert (NIH/NIAID) [E];Paules, Catharine (NIH/NIAID) [E]
Subject: RE: FDAAA lecture

My FDAAA Lecture will be the 5 Presidents Lecture: "Emerging and Re-Emerging Infectious Diseases: From AIDS to Zika". It is now 1st FINAL in the OD folder and you need to replace one slide with one that I have suggested. I will likely delete a few more slides before I make it "FINAL FINAL". Thanks.

From: Marston, Hilary (NIH/NIAID) [E]
Sent: Wednesday, April 25, 2018 11:50 AM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Folkers, Greg (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6) Doepel, Laurie (NIH/NIAID) [E] (b)(6) Eisinger, Robert (NIH/NIAID) [E] (b)(6) Paules, Catharine (NIH/NIAID) [E] (b)(6)
Subject: FDAAA lecture

The FDAAA folks would like a few sentences on your lecture topic (the upcoming May 4th lecture). I drafted versions for both 5 presidents and biomedical preparedness – which would you prefer and do you have edits for that text?

Emerging and Re-emerging Infectious Diseases: From AIDS to Zika
Presidential administrations face multiple unexpected crises, and global pandemics are among the most challenging. The lecture will trace the history of the NIH response to emerging and re-emerging infectious diseases, beginning with the earliest recognition of HIV through to the recent Zika epidemic in the Americas.

Or

The Challenge of Pandemic Preparedness
Having faced multiple pandemics and pandemic threats in recent decades, the U.S. government and its international partners have redoubled preparedness efforts. Preparedness done well is a multifaceted endeavor, including global surveillance networks, health care infrastructure ranging from primary care centers to referral hospitals, health care workforce capacity, and engagement with affected communities. Biomedical research, including the development of countermeasures, play a central role. The lecture will trace improvements in these areas, with a specific focus on vaccines.

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Wednesday, April 25, 2018 10:24 AM
To: Bennett, Arthur (NIH/NIAID) [E] (b)(6)

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Cc: Marston, Hilary (NIH/NIAID) [E] (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6)
Subject: Re: Fauci

Yes.

Adding Hilary here for her assistance.

Sent from my iPhone

On Apr 25, 2018, at 10:18 AM, Bennett, Arthur (NIH/NIAID) [E] (b)(6) wrote:

Can you send me a sentence or two about the topic of his lecture?

Arthur Bennett
Director
Office of Ethics, OD, NIAID
5601 Fishers Lane, Room 6C23
MSC 9811
Rockville, MD 20852
(b)(6)
fax (301) 402-8178

****
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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Wednesday, April 25, 2018 10:07 AM
To: Bennett, Arthur (NIH/NIAID) [E] (b)(6)
Subject: Re: Fauci

Thx

Sent from my iPhone

On Apr 25, 2018, at 10:05 AM, Bennett, Arthur (NIH/NIAID) [E] (b)(6) wrote:

Good news – just got off the phone with Mr. Saylor – the sole item they'll present to Dr. Fauci is an acrylic plaque valued about $50, so no "gift" at all (this is considered an item of little intrinsic value) and so I'll just get an Official Duty Activity memo ready so there's something in the files. This will be quick!

Arthur Bennett
Director

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Wednesday, April 25, 2018 9:54 AM
To: Bennett, Arthur (NIH/NIAID) [E] (b)(6)
Subject: RE: Fauci

Ok thx

Patricia L. Conrad
Special Assistant to the Director

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From: Bennett, Arthur (NIH/NIAID) [E]
Sent: Wednesday, April 25, 2018 9:53 AM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: RE: Fauci

Actually, it's not FDA, but an alumni association, so it's not government. I have to find out a few things to figure out what paperwork, if any, to file. I'll let you know asap.

Art

Arthur Bennett
Director
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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Wednesday, April 25, 2018 9:46 AM
To: Bennett, Arthur (NIH/NIAID) [E] (b)(6)
Subject: FW: Fauci
Importance: High

ASF will receive this award from FDA - see below and attached. We just heard about it on April 17th and received the letter today. I presume since it is FDA they are aware of what they can give ASF……

Do we need any paperwork for this. Feel free to contact Allen Sayler directly with questions.

thx

Patricia L. Conrad
Special Assistant to the Director

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From: Allen Sayler (b)(6)
Sent: Wednesday, April 25, 2018 8:51 AM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6) Marie Urban (b)(6)
Cc: Folkers, Greg (NIH/NIAID) [E] (b)(6) Zoon, Kathryn (NIH/NIAID) [V] (b)(6)
Subject: RE: Fauci
Importance: High

Patricia, my apologies as the letter was sent internally to the FDAAA leadership for signature and sending to you and Dr. Fauci. I have taken it upon myself to sign the letter and it is attached. I also

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want to set some time aside in the next few days to talk directly with Dr. Fauci to provide some ideas as well as answer any questions he might have regarding the ½ hour lecture from 8:30 am – 9:00 am on the morning of Friday, May 4th. I have copied some information related to the substance of the lecture to allow him to begin preparing remarks.

Please feel free to call me if you have any questions. Thank you.

The Harvey W. Wiley Lecture

Lecturers shall be prominent figures from the fields of public health, health research, public health education, health care delivery, science journalism, food and drug law, and public policy-making, who can:

- Promote broader public enlightenment of and support for the mission of FDA;
- Provide intellectually stimulating, futurist perspectives on 21st century roles of the FDA in public health;
- Initiate a meaningful dialogue on innovative ways the FDAAA and its membership can impact the current and future mission of the FDA and its capacities to protect and promote the public health.

A lectureship named in honor of Dr. Harvey W. Wiley, the renowned physician-chemist who, at the turn of the 20th century, championed a legislative crusade against food adulteration, earning him the title of "Father of the Pure Food and Drugs Act" when it was enacted into law in 1906. The Lecture will be presented at the FDLIL Annual Meeting in May

Mr. Allen R. Sayler, Senior Director of Food Consulting Services
EAS Consulting Group LLC,1700 Diagonal Road, Suite 750,Alexandria VA, 22314
+1 877-327-9808 (toll free) (b)(6) (direct) (b)(6) (mobile) 703-548-3270 (fax)
Email: (b)(6) www.easconsultinggroup.com

From: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, April 25, 2018 8:42 AM
To: Marie Urban (b)(6)
Cc: Allen Sayler (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6) Zoon, Kathryn (NIH/NIAID) [V] (b)(6)
Subject: RE: Fauci

Good morning:

We have not received the letter or any information about the event from Mr. Sayler. If I have missed an earlier email I apologize.
If there is a lecture that Dr. Fauci needs to give as part of the ceremony – we need to know the details as to length so that we can begin to prepare his remarks. Any information would be greatly appreciated.

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Thank you so much.

Patricia L. Conrad
Special Assistant to the Director

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From: Marie Urban (b)(6)
Sent: Tuesday, April 17, 2018 11:45 AM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Allen Sayler (b)(6)
Subject: Re: Fauci

Hi. Yes, Dr. Fauci has been selected to receive the FDA Alumni Association's Wiley Award. As part of this, he will attend the FDLI meeting on May 4 to speak from 8:30 to 9 AM. **Allen should be sending a letter confirming this with the location, time etc**. Could you send Allen a picture of Dr. Fauci and bio.? Dr. Fauci has worked with FDA so he may want to address his public health accomplishments and what he sees in the future. Thanks Patricia for your help. Marie

---

From: "Conrad, Patricia (NIH/NIAID) [E]" (b)(6)
Date: Tuesday, April 17, 2018 at 11:19 AM
To: Marie Urban (b)(6)
Subject: RE: Fauci

So just to confirm…he has won the award for sure?

And please also confirm – I should send Allen Sayler the bio and photo?

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Patricia L. Conrad
Special Assistant to the Director

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From: Marie Urban (b)(6)
Sent: Tuesday, April 17, 2018 10:21 AM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: FW: Fauci

Hi Patricia. I should have included you ,in this email. Marie

---

From: Marie Urban (b)(6)
Date: Tuesday, April 17, 2018 at 8:58 AM
To: Allen Sayler (b)(6) "Zoon, Kathryn (NIH/NIAID) [V]" (b)(6)
Cc: "Fredda Valenti (Shere)" (b)(6)
Subject: Fauci

Hi Allen. This is the nomination which could serve as a bio.

Kathy - Dr. Fauci has been selected by the FDAAA as the FDAAA Wiley Award recipient. They will formally send him a letter soon. He would be the speaker at the FDLI meeting on May 4. He should be there at 8 am and will speak. **(Allen what is the address of the meeting and time allotted for a presentation).** The attached nomination could serve as a bio unless his assistant has something else. Also, can they send a picture of Dr. Fauci? I am (b)(6) so can his assistant get this information to Allen Sayler (email address above with a cc to Fredda and me). Thanks Kathy, Marie

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From: Chertow, Daniel (NIH/CC/CCMD) [E]
Sent: Sat, 28 Apr 2018 17:48:30 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Perspective in Science
Attachments: 381.full.pdf, ATT00001.htm

Published yesterday.

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These polymers are semicrystalline and possess mechanical properties that compare well with those of polylactide.

Furthermore, the authors found that by blending two enantiomerically pure polymers of opposite stereochemistry in a 1:1 stoichiometric ratio, superior materials could be obtained. Because of a phenomenon known as stereocomplexation (*13*), the materials that result from the simple blending process have melting temperatures that are more than 75°C higher than those of either constituent polymer, potentially enabling use in high-temperature applications.

Plastics will continue to be critical for addressing the continuing demands of our society. New polymeric materials will, for example, be needed for energy generation and storage, to address healthcare needs, for food conservation, and for providing clean water. The circular materials economy will require implementation of an appropriate infrastructure to underpin collection and sorting of plastic that has reached the end of its first life before it reaches the environment (*4*). Beyond the design of new materials, this will require collaboration across scientific and nonscientific disciplines as well as political and public will to ensure success.

Studies such as that of Zhu *et al.*, in which disposed plastics can be infinitely recycled without deleterious effects on their properties, can lead to a world in which plastics at the end of their life are not considered as waste but as raw materials to generate high-value products and virgin plastics. This will both incentivize recycling and encourage sustainability by reducing the requirement for new monomer feedstocks. Current chemical recycling processes are expensive and energetically unfavorable, and further advances in monomer and polymer development and catalyst design are required to facilitate the implementation of economically viable sustainable polymers (*14*). ■

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10.1126/science.aat4997

VIROLOGY

Next-generation diagnostics with CRISPR

CRISPR-Cas biology promises rapid, accurate, and portable diagnostic tools

By **Daniel S. Chertow**

Rapid and accurate identification of infectious diseases is essential to optimize clinical care and guide infection control and public health interventions to limit disease spread both in highly specialized medical centers and remote health care settings. The ideal diagnostic test would be inexpensive, accurate, and provide a result rapidly, allowing for point-of-care use on multiple specimen types without need for technical expertise, ancillary equipment, or power. Such a test for highly pathogenic viruses that emerge in remote settings but might spread globally (for example, Ebola virus and Middle East respiratory syndrome coronavirus) would aid in early case detection and isolation, limiting disease spread and facilitating timely care (*1*). The sentinel discovery that prokaryotes (bacteria and archaea) have heritable adaptive immunity mediated through CRISPR and CRISPR-associated (Cas) proteins has led to transformative advances in molecular biology, most notably in gene editing (*2*). On pages 436, 444, and 439 of this issue, Chen *et al.* (*3*), Myhrvold *et al.* (*4*), and Gootenberg *et al.* (*5*), respectively, highlight how evolving insights into CRISPR-Cas biology are also revolutionizing the field of molecular diagnostics for infectious diseases, through detection of Zika virus (ZIKV), Dengue virus (DENV), and human papillomavirus (HPV) in human samples, and noninfectious diseases, such as detection of gene mutations in circulating cell-free DNA from lung cancer patients.

Prokaryotes store genetic elements from infectious agents (phages, plasmids, or transposons) in genomic loci called CRISPR arrays as memories for adaptive immunity. Cas proteins facilitate adaptive immunity through the processes of adaptation, CRISPR RNA (crRNA) generation, and interference (*6*). During adaptation, foreign genetic material is processed and selected for integration into the CRISPR array, providing a recall element during recurrent infection. Pre-crRNA is transcribed as a long precursor and processed into mature form as crRNA to guide Cas proteins to cleave complementary sequences of foreign elements (interference) to degrade and eliminate those elements. By uncovering the structural and functional components of these diverse systems, new tools, including those applicable to molecular diagnostics, are emerging (see the figure).

Chen *et al.* report the discovery that when CRISPR-Cas12a proteins cleave double-stranded DNA (dsDNA) in a sequence-specific manner, they induce robust nonspecific single-stranded DNA (ssDNA) trans-cleavage. The authors apply this observation to develop a rapid and accurate test to detect carcinoma-associated HPV types 16 and 18 from clinical specimens. HPV dsDNA is extracted from anal swabs and amplified through isothermal preamplification by recombinase polymerase amplification (RPA) (*7*), a method that is rapid and does not require specialized equipment. A Cas12a-crRNA complex binds to and cleaves target HPV dsDNA, which activates trans-cleavage of ssDNA. A fluorescent reporter coupled to ssDNA generates a fluorescent signal upon cleavage. This new approach, called DNA endonuclease-targeted CRISPR trans reporter (DETECTR), offers a promising platform for rapid and accurate detection of cervical cancer–associated HPV subtypes that, consistent with World Health Organization recommendations, might augment screening programs worldwide (*8*).

> ***"Future work will expand upon the range of diagnostic applications for infectious and noninfectious diseases..."***

Myhrvold *et al.* introduce a new approach to release and protect from degradation viral nucleic acids from clinical specimens, bypassing the need for nucleic acid extraction in molecular diagnostics. This method,

Critical Care Medicine Department, NIH Clinical Center, and the Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA. Email: chertowd@cc.nih.gov

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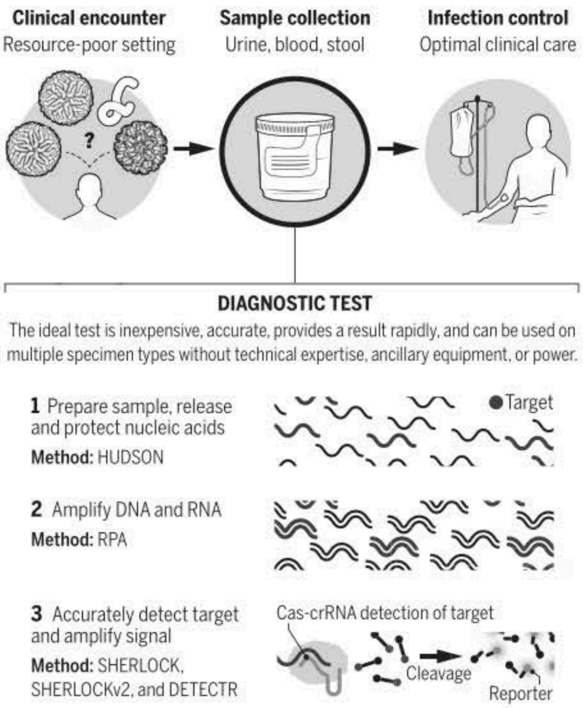
called HUDSON (heating unextracted diagnostic samples to obliterate nucleases), is a process of heat and chemical reduction that inactivates the high amounts of ribonucleases (RNases) found in body fluids and then lyses viral particles by disrupting the viral envelope, thereby releasing nucleic acids into solution. The authors combine HUDSON with SHERLOCK (specific high-sensitivity enzymatic reporter unlocking), a Cas13-based nucleic acid detection platform described previously (*9*). Similar to DETECTR, SHERLOCK combines RPA and an RNA-guided Cas13 that induces collateral cleavage of nucleic acids. In SHERLOCK, RNA coupled to a fluorescent reporter is cleaved, producing a fluorescent signal that is amplified though enzymatic activity, which enhances test sensitivity. By combining HUDSON and SHERLOCK, the authors develop a sensitive and specific diagnostic platform to detect the flaviviruses DENV and ZIKV directly from body fluids (urine, saliva, serum, plasma, and whole blood), with limited sample preparation or equipment, that provides a result within 1 to 2 hours. DENV and ZIKV cocirculate in many areas of Central and South America and have similar clinical presentation (*10*). ZIKV infection during pregnancy predisposes to severe congenital anomalies and is sexually transmissible, emphasizing the need for accurate diagnosis among pregnant women and their sexual partners (*11*). HUDSON combined with SHERLOCK reliably differentiates between DENV, ZIKV, and another flavivirus, yellow fever virus (YFV), which cocirculate and cause severe parallel epidemics in Brazil (*10*). The platform can also reliably distinguish between four closely related DENV serotypes and can detect single-nucleotide polymorphisms (SNPs) among ZIKV isolates. This could be applied to detect SNPs that confer antimicrobial resistance [for example, among *Mycobacterium tuberculosis* (TB) isolates] or enhance pathogen virulence or transmissibility [for example, among highly pathogenic avian influenza A (H5N1) viruses], allowing for better tracking of emerging pathogens. Finally, the authors show that the fluorescent readout of SHERLOCK can be replaced with a visual readout on a paper test-strip, suitable for point-of-care field application.

Gootenberg *et al.* introduce SHERLOCK version 2 (v2). This improved assay allows for detection of three ssRNA targets and one dsDNA target in a single reaction. The authors biochemically characterize 17 CRISPR-Cas13a and -Cas13b enzymes, selecting three with distinct cleavage preferences, that when combined with a Cas12a enzyme and RPA accurately detect ZIKV ssRNA, synthetic ssRNA, DENV ssRNA, and synthetic dsDNA by visual readout in less than 90 minutes. A potential application of multitarget RNA and DNA detection by SHERLOCKv2 could be a rapid and accurate diagnostic test for pneumonia pathogens. DNA and RNA viruses alone or in combination with bacterial infection cause pneumonia, a leading killer of children worldwide (*12*). An accurate and affordable point-of-care diagnostic for pneumonia would allow for early and targeted use of antibiotics in remote settings.

Another feature of SHERLOCKv2 is quantitative and sensitive target detection. This could be applied to a portable and accurate test to monitor viral load among HIV patients receiving antiviral therapy in resource-limited settings, substantially improving global HIV care (*13*). SHERLOCKv2 was also used to detect mutations in cell-free DNA from the blood of non–small cell lung cancer patients by fluorescence- and lateral flow–based readouts, further expanding the potential applications to liquid biopsy. Finally, in proof-of-concept in vitro experiments, the authors successfully apply SHERLOCKv2 both as a gene-editing therapeutic that corrects a gene mutation that predisposes to colon cancer, and as a diagnostic to concurrently determine the proportion of genes successfully edited.

Application of CRISPR diagnostics

Next-generation diagnostics applying CRISPR-Cas biology will facilitate early disease detection and intervention.



These emerging diagnostic tools will by necessity be compared to standard diagnostics to ensure sensitivity and specificity and will need to be field-tested to guarantee performance in patient care settings, as environmental conditions and end-user application might affect performance. Proven assays, if affordable, promise to improve care in resource-limited settings where undifferentiated febrile illness is the norm and where gaps or delays in diagnosis, targeted care, and infection control contribute to infectious disease mortality and spread. For example, TB results in an estimated 1.3 million deaths annually, the leading cause from a single infectious agent, and most deaths could be prevented with early diagnosis and treatment (*14*). Assays might be expanded to provide insight into pathogen resistance patterns to guide antimicrobial therapy, molecular correlates of pathogen viability to guide infection control, and compatibility with additional specimen types such as stool, respiratory secretions, and cerebrospinal fluid, that when integrated with clinical judgment might for example, differentiate etiology of enteritis, pneumonia, and meningitis. Future work will expand upon the range of diagnostic applications for infectious and noninfectious diseases in the clinic, laboratory, and field where assay accuracy, reliability, simplicity, speed, flexibility, and cost will determine the scope of impact. ■

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Science

Next-generation diagnostics with CRISPR

Daniel S. Chertow

Science **360** (6387), 381-382.
DOI: 10.1126/science.aat4982

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From: Grady, Christine (NIH/CC/BEP) [E]
Sent: Sat, 28 Apr 2018 14:33:29 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: FW: Update RE: UMSON Reunion 2018 CNE lectures and presentations
Importance: High

To print agenda and maybe some notes

From: Sikorski, Cynthia (b)(6)
Sent: Friday, April 27, 2018 5:02 PM
To: Jennifer Wenzel (b)(6) Mikaela Olsen (b)(6) (b)(6) (b)(6) (b)(6) Ogbolu, Yolanda (b)(6) Milburn, Trish (b)(6) Fornili, Katherine (b)(6) (b)(6) Grady, Christine (NIH/CC/BEP) [E] (b)(6)
Subject: Update RE: UMSON Reunion 2018 CNE lectures and presentations
Importance: High

Hi everyone,
Thank you again for agreeing to give a CNE lecture at UMSON's All Alumni Reunion tomorrow, Saturday, April 28, 2018. Here is the schedule for the day. As you can see, the lectures are back to back so please try to keep your presentation to about 45-50 minutes so that folks have a chance to get from one room to another. As I hope you know, you are welcome to attend all of the events as our guest: Continental Breakfast, Reunion Lunch and the Cocktail Reception. We are very grateful for your participation in Reunion 2018 and look forward to seeing you tomorrow:

9 am	**Registration**, continental breakfast, merchandise for sale.
10-11 am	**Reunion Tours**: Bench Research Lab, Simulation Labs, Museum, Chair Yoga Class (rm 211)
11 am	**Reunion Program** – **Dean Jane Kirschling** State of the School address; **Adrianna Wolff**, BSN Student speaker, Distinguished Alumni Award presentation to **Robin Newhouse, PhD '00, MS '99, BSN '87, RN, FAAN,** Dean, University of Indiana School of Nursing
12:30 pm	**Large Group Photo**, Main Lobby area
12:30-1:45 pm	**All Alumni Reunion Lunch**, UMB Campus Center. Group/Class photos
2-3 pm	**Session I, Rm 150** *Navigating Cancer Survivorship* **Jennifer Wenzel,** PhD, MS '00, RN, CCM, FAAN **MiKaela Olsen,** DNP (anticipated 2019), MS, APRN-CNS, AOCNS, FAAN
	Session I, Rm 140

AMERICAN OVERSIGHT

Creating and Sustaining Healthy Workplace Environments

Dorrie Fontaine, PhD, MS '77, RN, FAAN
Cheryl L. Cioffi, DNP '11, MS '00, BSN '99, RN, ANP-BC, NEA-BC, FACHE

3-4 pm **Session II, Room 140**
One Step Forward, Two Steps Back: Overview of Current U.S. Drug Policy
Katherine Fornili, DNP '16, MPH, RN, CARN, FIAAN

Session II, Room 150
Global Health: Nursing Leadership and Implications
Yolanda Ogbolu, PhD '11, MS '05, BSN '04, RN, CRNP-Neonatal, FAAN
Adanna Agbo, DrPH(c), MPH, MS '07, RN, PHNA-BC
Trish Milburn, DNP '12, MS '93, CRNP

4-5 pm **Keynote Lecture, Room 140**
Ethics Then and Now: Contributions of Nurse Bioethicists
Christine Grady, PhD, RN, FAAN

5 – 6:30 pm **Alumni Networking Reception,** Student Services area

Cynthia Sikorski
Senior Associate Director of Alumni Relations
University of Maryland School of Nursing
655 W. Lombard Street, Suite 516
Baltimore, MD 21201
410-706-0674
(b)(6) (cell)
(b)(6) (note that this is my new email address)

Save the date for UMSON's ALL ALUMNI REUNION – Saturday, April 28, 2018!
RSVP online at: https://cf.umaryland.edu/checkout/son/2018-alumni-reunion/

AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Fri, 27 Apr 2018 18:00:12 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: 2018 National Ryan White Conference - plenary
Attachments: FW: Invitation to speak at the 2018 National Ryan White Conference on HIV Care and Treatment, FW: Invitation to speak at the 2018 National Ryan White Conference on HIV Care and Treatment, RE: Invitation to speak at the 2018 National Ryan White Conference on HIV Care and Treatment, FW: Invitation to speak at the 2018 National Ryan White Conference on HIV Care and Treatment, FW: 2018 National Ryan White Conference on HIV Care and Treatment - on behalf of Dr Fauci

AMERICAN OVERSIGHT

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Fri, 27 Apr 2018 17:59:54 +0000
To: Conrad, Patricia (NIH/NIAID) [E]
Subject: FW: Invitation to speak at the 2018 National Ryan White Conference on HIV Care and Treatment

Patricia L. Conrad
Special Assistant to the Director

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Thursday, April 26, 2018 7:44 PM
To: NIAID OD AM (b)(6)
Subject: FW: Invitation to speak at the 2018 National Ryan White Conference on HIV Care and Treatment

Let us discuss. I should do this. I could combine discussion of scientific issues with policy to "end the pandemic".

From: Cheever, Laura (HRSA)
Sent: Thursday, April 26, 2018 5:02 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Subject: Invitation to speak at the 2018 National Ryan White Conference on HIV Care and Treatment

Dr. Fauci,

Thank you so much for taking the time to meet with me earlier this month. I appreciate your advice.

As we discussed at that meeting, attached is the invitation to speak at the opening plenary of the 2018 National Ryan White Conference on HIV Care and Treatment to be held December 11-14, 2018. I hope you will be able to join the conference.

Laura

Laura W. Cheever, MD, ScM

AMERICAN OVERSIGHT

Associate Administrator | HIV/AIDS Bureau | Health Resources and Services Administration | Department of Health and Human Services | 5600 Fishers Lane, 9W37, Rockville, Maryland 20857 | (b)(6) | (b)(6) (b)(6) direct) | www.hab.hrsa.gov

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AMERICAN OVERSIGHT

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Fri, 27 Apr 2018 17:55:27 +0000
To: Cheever, Laura (HRSA)
Subject: FW: Invitation to speak at the 2018 National Ryan White Conference on HIV Care and Treatment

Hi Laura –

Dr. Fauci asked that I respond on his behalf – he would be happy to speak at the 2018 National Ryan White conference. If it is at all possible – would Dr. Fauci be able to speak earlier in the day on December 11th – perhaps in the morning at 10:00 am? If not – that is fine and we will work it out but if it is at all possible for him to speak earlier – we would greatly appreciate it.

Many thanks,
-patty

Patricia L. Conrad
Public Health Analyst and
Special Assistant to the Director
National Institute of Allergy and Infectious Diseases
The National Institutes of Health
31 Center Drive, MSC 2520 - Room 7A03
Bethesda, Maryland 20892
(b)(6)
301-496-4409 fax

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AMERICAN OVERSIGHT

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Mon, 7 May 2018 18:49:49 +0000
To: Cheever, Laura (HRSA)
Cc: Matosky, Marlene (HRSA);Hauck, Heather (HRSA);Barney, Kristina (HRSA)
Subject: RE: Invitation to speak at the 2018 National Ryan White Conference on HIV Care and Treatment

Hi Laura –
Dr. Fauci will be able to participate at 3 pm now. Please let us know how long you would like him to speak for and if there will be a Q and A. He will use powerpoint slides.

Thank you.

Patricia L. Conrad
Special Assistant to the Director

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From: Cheever, Laura (HRSA)
Sent: Thursday, May 03, 2018 9:14 AM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Matosky, Marlene (HRSA) (b)(6) Hauck, Heather (HRSA) (b)(6) Barney, Kristina (HRSA) (b)(6)
Subject: RE: Invitation to speak at the 2018 National Ryan White Conference on HIV Care and Treatment

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Laura W. Cheever, MD, ScM

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To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Vance, Meaghan (NIH/NIAID) [C] (b)(6)
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Laura

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Associate Administrator | HIV/AIDS Bureau | Health Resources and Services Administration | Department of Health and Human Services | 5600 Fishers Lane, 9W37, Rockville, Maryland 20857 | (b)(6) | (b)(6) (b)(6) direct) | www.hab.hrsa.gov

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Mon, 7 May 2018 19:43:01 +0000
To: Greg Folkers (b)(6) Meaghan (NIH/NIAID) [C]
Subject: FW: Invitation to speak at the 2018 National Ryan White Conference on HIV Care and Treatment

For the folder pls

Patricia L. Conrad
Special Assistant to the Director

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From: Cheever, Laura (HRSA)
Sent: Monday, May 07, 2018 3:21 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Matosky, Marlene (HRSA) (b)(6) Hauck, Heather (HRSA) (b)(6) Barney, Kristina (HRSA) (b)(6)
Subject: RE: Invitation to speak at the 2018 National Ryan White Conference on HIV Care and Treatment

Thanks. No Q&A. We'll get the details of the time, etc. to you in a longer communication once we've finalized the agenda. L.

Laura W. Cheever, MD, ScM

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Monday, May 07, 2018 2:50 PM
To: Cheever, Laura (HRSA) (b)(6)
Cc: Matosky, Marlene (HRSA) (b)(6) Hauck, Heather (HRSA) (b)(6) Barney, Kristina (HRSA) (b)(6)
Subject: RE: Invitation to speak at the 2018 National Ryan White Conference on HIV Care and Treatment

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Sent: Thursday, May 03, 2018 9:14 AM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Matosky, Marlene (HRSA) (b)(6) Hauck, Heather (HRSA) (b)(6)
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-patty

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From: Cheever, Laura (HRSA)
Sent: Thursday, April 26, 2018 5:02 PM

AMERICAN OVERSIGHT

To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Vance, Meaghan (NIH/NIAID) [C] (b)(6)
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AMERICAN OVERSIGHT

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Thu, 8 Nov 2018 20:16:37 +0000
To: Vance, Meaghan (NIH/NIAID) [C]
Subject: FW: 2018 National Ryan White Conference on HIV Care and Treatment - on behalf of Dr Fauci

For the folder.

Patricia L. Conrad
Public Health Analyst and
Special Assistant to the Director
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31 Center Drive, MSC 2520 - Room 7A03
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From: Matosky, Marlene (HRSA) (b)(6)
Sent: Thursday, November 8, 2018 3:08 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6) Cheever, Laura (HRSA) (b)(6)
Cc: Hauck, Heather (HRSA) (b)(6) Barney, Kristina (HRSA) (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6)
Subject: RE: 2018 National Ryan White Conference on HIV Care and Treatment - on behalf of Dr Fauci

Thank you for following up. I've attached our current run of show for the plenary panel. The run of show may change slightly depending on the Secretary's schedule. Dr. Fauci has up to 30 minutes to provide his remarks. We would like to receive Dr. Fauci's slides a few days ahead of the plenary panel. The plenary panel starts at 3pm on Tuesday, December 11, 2018. The address is below. We ask the speakers to arrive by 2:30pm at the facility. We can meet Dr. Fauci in the lobby sitting area and escort him to the green room. Cheever reached out to Meaghan Vance for a high resolution headshot and 200 word bio earlier this week.

My cell is (b)(6) You are welcome to call or text me as needed. We are looking forward with excitement to Dr. Fauci's participation. Thank you.

Facility address:
Gaylord National Harbor Hotel and Convention Center

AMERICAN OVERSIGHT

201 Waterfront Street, Oxon Hill, MD 20745.

Marlene Matosky, MPH, RN
Chief – Clinical and Quality Branch
Division of Policy and Data | HIV/AIDS Bureau | Health Resources and Services Administration
5600 Fishers Lane, Rm 09N154 | Rockville, MD 20857 | PH: (b)(6) | FAX: 301-443-8143 |
(b)(6)

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Thursday, November 08, 2018 12:49 PM
To: Cheever, Laura (HRSA) (b)(6)
Cc: Matosky, Marlene (HRSA) (b)(6) Hauck, Heather (HRSA) (b)(6)
Barney, Kristina (HRSA) (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6)
Subject: 2018 National Ryan White Conference on HIV Care and Treatment - on behalf of Dr Fauci

Good afternoon:

I am touching base to see if you have any further details regarding Dr. Fauci's participation at your event as we would like to start to make preparations for his remarks.

If there is a detailed agenda you can send we would greatly appreciate it. Also – we would need to know how long you would like him to speak for.

Many thanks,
-patty

Patricia L. Conrad
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Cc: Matosky, Marlene (HRSA) (b)(6) Hauck, Heather (HRSA) (b)(6)
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AMERICAN OVERSIGHT

From: Paules, Catharine (NIH/NIAID) [E]
Sent: Fri, 27 Apr 2018 18:04:38 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: RE: Request from AAI

Ive reached out to Ellen and cced Patty and Greg. Will let you know. Thanks, Cat

---

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Friday, April 27, 2018 1:48 PM
To: Paules, Catharine (NIH/NIAID) [E] (b)(6)
Subject: RE: Request from AAI
Importance: High

Cat:
I have not heard anything further from Ellen Fox or anyone else at the AAI. Would you please get back to them and see if there is any further information on this project. Also, what is the date that this is due.
Thanks,
Tony

From: Paules, Catharine (NIH/NIAID) [E]
Sent: Friday, April 27, 2018 12:42 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Folkers, Greg (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: FW: Request from AAI
Importance: High

Hi Dr. Fauci,
This is a follow up to our discussion on the way to rounds about upcoming writing projects. This is the communication with the Journal of Immunology regarding writing the opening commentary for a special issue. We spoke with Ellen Fox via phone and she had said she would get us a list of topics/authors (I do not believe she has sent that). Once we had that list we were planning to pick a third author with a strong influenza immunology background (potentially Adrian Mcdermott). We had thrown some ideas around regarding imprinting etc as topics but hadn't officially decided on topics. Plan was for a prebrief once we heard back from Ellen. Note deadline to submit is September 1. Let me know if you need anything else, thanks, Cat

---

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Wednesday, March 28, 2018 4:26 PM
To: 'Hogan, Michele' (b)(6)
Cc: Gene Oltz (b)(6) (b)(6) Wayne M. Yokoyama (b)(6) JoAnne Flynn (b)(6) (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: RE: Request from AAI

AMERICAN OVERSIGHT

Michele:
Thanks for the note. I already spoke to Ellen Fox and confirmed that I would do this.
Best regards,
Tony

From: Hogan, Michele (b)(6)
Sent: Tuesday, March 27, 2018 6:25 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Gene Oltz (b)(6) (b)(6) Wayne M. Yokoyama (b)(6) JoAnne Flynn (b)(6) (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: Request from AAI

Dear Tony:

Attached please find our invitation regarding a special issue of *The Journal of Immunology*.

With best regards,
Michele

M. Michele Hogan, Ph.D.
Executive Director
The American Association of Immunologists
1451 Rockville Pike, Suite 650
Rockville, MD 20852
(b)(6)
www.aai.org

AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Fri, 27 Apr 2018 17:43:55 +0000
To: Bruce Gellin
Subject: RE: Grand Challenge for Universal Flu Vaccine development_FINAL.pdf

Thanks, Bruce.

From: Bruce Gellin (b)(6)
Sent: Friday, April 27, 2018 9:14 AM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: Grand Challenge for Universal Flu Vaccine development_FINAL.pdf

Tony:

I assume you are aware but wanted to be sure. Embargoed until 11:30.

Bill Gates will announce this later this morning in his Shattuck Lecture in Boston.

Best,

Bruce

AMERICAN OVERSIGHT

From: Paules, Catharine (NIH/NIAID) [E]
Sent: Fri, 27 Apr 2018 16:41:54 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Folkers, Greg (NIH/NIAID) [E];Vance, Meaghan (NIH/NIAID) [C];Conrad, Patricia (NIH/NIAID) [E]
Subject: FW: Request from AAI
Attachments: Invitation from AAI (002).pdf
Importance: High

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Cc: Gene Oltz (b)(6) (b)(6) Wayne M. Yokoyama (b)(6) JoAnne Flynn (b)(6) (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: RE: Request from AAI

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Subject: Request from AAI

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Michele

AMERICAN OVERSIGHT

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Executive Director
The American Association of Immunologists
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Rockville, MD 20852
(b)(6)
www.aai.org

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Mark M. Davis, Ph.D.

Executive Director
M. Michele Hogan, Ph.D.

March 27, 2018

Anthony S. Fauci, M.D.
Director
National Institute of Allergy and Infectious Diseases
31 Center Drive, MSC 2520
Bethesda, MD 20892-2520

Dear Dr. Fauci:

As you likely know, in 2017 *The Journal of Immunology (The JI)* launched a series of exciting and timely topical review issues. These issues featured 8–10 invited articles from the field's top scientists. The first issue focused on the microbiome (http://www.jimmunol.org/microbiome), and the second was on cancer immunotherapy (http://www.jimmunol.org/cancerimmunotherapy). Both of these special issues were resoundingly successful, and we intend to continue this initiative.

We are now planning our 2019 topical issue, tentatively entitled "Immunity to Influenza: A Moving Target", and we invite you to write a commentary that will be featured as an introductory keynote for the entire issue. We know our readership would appreciate your perspective on this important health challenge, including such topics as: (i) flu biology and immune responses, especially the critical role of basic immunology research in understanding these processes; (ii) vaccine development — lessons learned (or not learned); and (iii) challenges and opportunities for the future (immediate or far-reaching). Of course, we defer to your deep understanding of the field as to the precise topics you would like to highlight.

As an AAI member and the nation's most authoritative voice on the importance of immunological research, you would provide an invaluable perspective for the scientific and lay communities. We would be extremely honored if you would agree to author this commentary.

As the topical review issue will be published in January 2019, the initial submission deadline is September 1, 2018. If you are able to accept this invitation, please let Dr. Eugene Oltz know directly (b)(6) or your staff may contact Dr. Ellen Fox, AAI Science Associate (b)(6) or (b)(6)-(b)(6)

1451 ROCKVILLE PIKE, SUITE 650, ROCKVILLE, MARYLAND 20852
PHONE: (b)(6) * FAX: 301-634-7887
E-MAIL: INFOAAI@AAI.ORG * WEB: WWW.AAI.ORG

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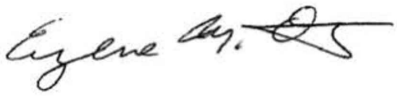
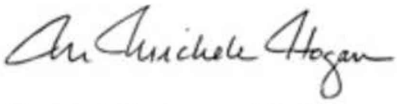
We would be glad to answer any questions you might have as to content, length, scheduling, logistics, or format. We also will inform you of other authors who will contribute more focused reviews once they have been finalized.

We are very excited about continuing this initiative for *The JI* and look forward to hearing from you.

Sincerely,



Eugene Oltz, Ph.D.
Incoming Editor-in-Chief, *The Journal of Immunology*
Professor of Pathology & Immunology
Washington University School of Medicine



M. Michele Hogan, Ph.D.
Executive Director and
Executive Editor, *The Journal of Immunology*
The American Association of Immunologists

Wayne M. Yokoyama, M.D.
President, The American Association of Immunologists
Sam J. and Audrey Loew Levin Professor of Medicine
Professor of Pathology and Immunology, Division of Rheumatology
Washington University School of Medicine

JoAnne L. Flynn, Ph.D.
Vice President, The American Association of Immunologists
Professor, Department of Microbiology and Molecular Genetics
University of Pittsburgh School of Medicine

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AMERICAN OVERSIGHT

From: Mary Hollowell
Sent: Fri, 27 Apr 2018 15:29:48 +0000 (UTC)
To:
(b)(6) Anthony (NIH/NIAID) [E]
Cc:
(b)(6)
(b)(6) Hollowell;Mary
(b)(6)
(b)(6)
(b)(6)
(b)(6)
(b)(6)
(b)(6)
(b)(6)
(b)(6)
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(b)(6)
(b)(6)
(b)(6)
(b)(6)
Subject: universal vaccine coverage in Atlanta Journal Constitution

Dear Sheila Poole, Helena Oliviera, Alyssa Pointer, & Editors of the AJC:

This letter is written in response to your 4/26/18 column titled "More Details on the Universal Flu Vaccine" [p. A4]. As a mother & concerned citizen, I am sharing important research with you. The information is below, and it includes a vaccination study in Africa stating, "mortality was 5 times higher for DTP-vaccinated children" (p. 196 4.1). Other data was collected by Anthony R. Mawson or shared in the documentary film VAXXED. My own public comments on the subject are also included. In light of such data, the push for a universal flu vaccine is unacceptable! Please research and share.

Sincerely,
Mary Nix Hollowell

(b)(6)
C (b)(6)

A nightmare flu season finally comes to an end: universal flu vaccine (subsection, bottom)

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https://www.myajc.com/lifestyles/health/nightmare-flu-season-finally-comes-end/PoxtB2Yvt3dAPsFzXxwmII/#

VAXXED
http://vaxxedthemovie.com

Mary Hollowell asks GA EPD about mercury & aluminum
https://youtu.be/67zV7rTXFWM

Pilot comparative study on the health of vaccinated and unvaccinated 6- to 12-year-old U.S. children
https://web.archive.org/web/20170504235436/http://oatext.com/pdf/JTS-3-186.pdf

The Introducation of Diptheria-Tetnus-Pertussis and Oral-Polio-Vaccine Among Young Infants in an Urban African Community
https://www.ebiomedicine.com/article/S2352-3964(17)30046-4/pdf

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Fri, 27 Apr 2018 13:36:52 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: FW: Input: 372243 Natsios (Snowcroft Invitation for Dr. Collins)
Attachments: 372243 Natsios #2 corr.pdf, 372243 Natsios #3 corr.pdf

What say you.....

Patricia L. Conrad
Special Assistant to the Director

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From: Harris, Kara (NIH/NIAID) [E]
Sent: Friday, April 27, 2018 8:47 AM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Billet, Courtney (NIH/NIAID) [E] (b)(6) Haskins, Melinda (NIH/NIAID) [E] (b)(6)
Subject: Input: 372243 Natsios (Snowcroft Invitation for Dr. Collins)

Hi, Patty –

Dr. Collins has received the attached invitation to be the keynote speaker at the 2nd Annual Pandemic and Biosecurity Policy conference sponsored by the Snowcroft Institute of International Affairs at the Bush School at Texas A&M University. The conference will focus on the coming threats from pandemic disease and international health security. It is being held on the morning of May 26, 2018, at the National Press Club in Washington, DC.

(b)(6) is requesting Dr. Fauci's input on whether he should accept or decline this invitation. They would like our response by 2:00 p.m. on Monday.

Thanks for your help!
Kara

Kara M. Harris, MPH
Section Chief for Controlled Correspondence and Public Inquiries
Office of Communications and Government Relations
National Institute of Allergy and Infectious Diseases
National Institutes of Health

AMERICAN OVERSIGHT

5601 Fishers Lane, Room 6F30
Bethesda, MD 20892
Phone: (b)(6)
Fax: (301) 402-3573
E-mail: (b)(6)

Disclaimer:
The information in this e-mail and any of its attachments is confidential and may contain sensitive information. It should not be used by anyone who is not the original intended recipient. If you have received this e-mail in error please inform the sender and delete it from your mailbox or any other storage devices. National Institute of Allergy and Infectious Diseases shall not accept liability for any statements made that are sender's own and not expressly made on behalf of the NIAID by one of its representatives.

AMERICAN OVERSIGHT

From: Natsios, Andrew S
Sent: Thu, 26 Apr 2018 14:42:20 +0000
To: Collins, Francis (NIH/OD) [E];Collins, Francis (NIH/OD) [E]
Cc: Gerald Parker;Beach, Sarah R;Blackburn, Christine C
Subject: Invitation to serve as keynote speaker at
Attachments: Collins Letter.pdf

Dr. Collins-

We invite you to serve as the keynote speaker at our 2nd Annual Pandemic and Biosecurity Policy conference held at the National Press Club in Washington, DC on May 16th. Your leadership in this important topic is vital to national health and security; we hope to stress the need for government support and action at our event. Your participation would be a highlight for the conference., Please see attached for our formal invitation.

Best wishes,
Andrew Natsios

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SCOWCROFT INSTITUTE ★★★
OF INTERNATIONAL AFFAIRS

The Bush School
OF GOVERNMENT & PUBLIC SERVICE
TEXAS A&M UNIVERSITY

Dr. Francis Collins
Director
National Institutes of Health
9000 Rockville Pike
Bethesda, Maryland 20892

April 17, 2018

Dear Dr. Collins:

The Scowcroft Institute of International Affairs at the Bush School at Texas A&M University invites you to be the keynote speaker at our 2nd Annual Pandemic and Biosecurity Policy Conference focusing on the coming threats from pandemic disease and international health security. The conference is being held on **May 16th from 8:45-11:00am** in the Holman Lounge at the National Press Club in Washington, DC.

Last year, we hosted the inaugural conference as a continuation of the Scowcroft Institute Pandemic Policy Summit, held every October in College Station, Texas. Both events bring major leaders, researchers, academics, and practitioners together from diverse backgrounds, including biodefense response organizations, U.S. Government institutions, multilateral and non-governmental organizations, private sector partners, developing countries, foundations, think tanks and institutions critical for improved pandemic response, biodefense, and health security.

This year's conference will be led by Andrew Natsios, Director of the Scowcroft Institute and former USAID Administrator, and moderated by Dr. Gerald Parker, the Associate Dean for Global One Health at Texas A&M's College of Veterinary Medicine and Director of the Pandemic and Biosecurity Policy Program at the Scowcroft Institute. We will hold two plenaries: one focused on Pandemic First Responders and one focused on Multi-Donor Perspectives on Pandemic and Health Security Response.

We hope you will join us on May 16, 2018, and we look forward to working with you and answering any questions you may have about the event or your planned participation.

Sincerely Yours,

Andrew Natsios

Andrew S. Natsios
Executive Professor and Director,
Scowcroft Institute for International Affairs
George H.W. Bush School of Government
& Public Service
Texas A&M University
College Station, TX

Gerald W. Parker

Dr. Gerald W. Parker Jr. DVM, PhD.
Associate Dean for Global One Health
College of Veterinary Medicine
Director of Pandemic and
Biosecurity Policy Program,
Scowcroft Institute for International Affairs
College Station, TX

We hope you can join us.

AMERICAN OVERSIGHT

From: Bruce Gellin
Sent: Fri, 27 Apr 2018 13:13:52 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Grand Challenge for Universal Flu Vaccine development_FINAL.pdf
Attachments: Grand Challenge for Universal Flu Vaccine development_FINAL.pdf, ATT00001.htm

Tony:

I assume you are aware but wanted to be sure. Embargoed until 11:30.
Bill Gates will announce this later this morning in his Shattuck Lecture in Boston.

Best,

Bruce

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Ending the Pandemic Threat: A Grand Challenge for Universal Influenza Vaccine Development

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  - Key dates and deadlines for this Grand Challenges 8
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The Grand Challenge

(Facilitating innovation requires understanding) how do we create ecosystems that promote creativity…that promote innovation of benefit to the world and to humanity.[1]
Eliane Ubalijoro, McGill University, GCE Grantee

The net effect of Grand Challenges will be a massive return – these investments, really, will be traceable to saving millions of lives.[2]
Bill Gates

This is an all-hands-on-deck time. If we get there, we can make influenza history.[3]
Bruce Gellin, Sabin Vaccine Institute

Background

2018 marks the 100-year anniversary of the most severe influenza pandemic in recorded history, which killed an estimated 50 million people worldwide – more than the total deaths caused by the First World War. The subsequent influenza pandemics of 1957, 1968, 1977, and 2009, though milder than the 1918 pandemic, demonstrated the potential of influenza viruses to cause excessive morbidity, mortality, and, more generally, severe disruptions of healthcare systems. Clearly, the threat of pandemic influenza is very real. Also, influenza viruses pose a significant threat to humankind, with seasonal influenza disease leading to an estimated 290,000 – 650,000 deaths each year.

While vaccination remains the best tool for prevention of disease, the current influenza vaccines significantly underperform compared to the effective and durable vaccines used against other vaccine-preventable diseases around the world. One key reason for this comparatively reduced effectiveness is the influenza virus' propensity for generating mutations in its surface antigens – the very targets of today's vaccines. Furthermore, the predominant technologies for influenza vaccine production necessitate a protracted and inefficient manufacturing cycle and a huge supporting mechanism for biennial flu vaccine strain recommendations; by the time vaccines are ready for distribution – 6+ months after strain determination – the viruses circulating during next season may not match up well with those strains in vaccines leading to less than optimal vaccine effectiveness. Although other factors also contribute to poor vaccine effectiveness, the annual formulation changes, the cost of and limited access to current influenza vaccines (regardless of how well matched they are to circulating strains), and need for annual vaccinations are barriers to protecting against global seasonal influenza, particularly among those in low- and middle-income countries. Furthermore, at best they may only offer partial or minimal protection against emerging pandemic strains. Clearly, there is an unmet public health need for a transformative, game-changing universal influenza vaccine that will protect against all influenza strains for longer duration, alleviating the need for annual formulations and vaccinations and leading to a panacea for tackling pandemic and seasonal influenza disease threats.

---

[1] Grand Challenges in Global Health – the First Ten Years
[2] Grand Challenges in Global Health – the First Ten Years
[3] Andrew Nusca, "Why We Need a Universal Flu Vaccine", *Fortune*, 2018

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Objectives

To find a game changing, universal solution to all these challenges, the Bill & Melinda Gates Foundation and the Page Family are launching the "Universal Influenza Vaccine Development Grand Challenge" during the centenary year of the 1918 flu pandemic. The goal of this Grand Challenge is to identify novel, transformative concepts that will lead to development of universal influenza vaccines offering protection from morbidity and mortality caused by all subtypes of circulating and emerging (drifted and shifted) Influenza A subtype viruses and Influenza B lineage viruses for at least three to five years. It is envisaged that such a universal influenza vaccine would address the threat from both seasonal and pandemic influenza, thus alleviating the need for annual seasonal influenza vaccination campaigns, averting significant global morbidity and mortality, and better preparing the world for the next influenza pandemic.

While other funders are supporting development of universal Influenza vaccines, three things set this Grand Challenge apart. We seek to fund ideas that are bold and innovative, bridging the funding 'valley of death' to translate these novel approaches into products ready for human clinical trials. We also aim to encourage interdisciplinary collaboration and cross-fertilization of ideas from outside the traditional influenza research community. Third, we seek completely transformative approaches rather than incremental research.

Our collective belief is that innovation is catalyzed through rigorous collaboration and enriching of ecosystems, and we hope this Grand Challenge will stimulate creative thinking beyond the traditional influenza community. Although not exhaustive, examples of researchers and disciplines we would like to see further integrated and supported include computational and systems biologists, virologists, immunologists, bioinformatics, artificial intelligence, deep learning, machine learning, the HIV/AIDS and cancer immunotherapy research communities, etc. Fundamentally, we are looking for unconventional approaches that effectively drive or harness immune responses in desired ways and **develop universal influenza vaccines that are ready to start clinical trials by 2021**.

Approach

All proposals must be aligned with the Gates Foundation's intervention Target Product Profile (iTPP). The iTPP (detailed below) describes the desired characteristics of a universal influenza vaccine. Most importantly, new vaccines should have the potential to be used in all age groups around the world, especially in developing countries. We are looking for affordable, effective vaccines that are suitable for delivery through existing immunization programs in-country, which has implications for product presentation and stability as well as for dosing route and schedule. The vaccines need to be broadly protective across Influenza A and B strains for a minimum of three to five years. Technologies will need to be scalable to meet worldwide demand.

We are looking for proposals that:

- Engage scientists across a variety of disciplines*, including those new to the influenza field*
- Demonstrate *innovative thinking* by incorporating concepts or technologies not currently being used within/addressed by the influenza vaccine field
- Present concepts and strategies that are "off the beaten track," significantly radical in conception, and daring in premise.

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Please note: grantees will have access to a wide-range of Gates Foundation-funded resources and technology platforms to support their projects.

Examples of what we're looking for may fall into broad categories:

1. **Antigen-centric:** discovering new antigens/targets through Artificial Intelligence, Machine Learning, and/or Deep Learning approaches to get beyond traditional surface hemagglutinin
2. **Host-centric:** approaches that (a) generate, enhance, or modify human immune protection, including sterilizing immunity (b) ensure longer term (possibly life-long) immune response (c) describe surrogates for longevity of immune response and (d) that target specific tissue or cell types for appropriate induction of local and systemic immunity leading to broader and longer protection
3. **Technology-centric**: including (a) novel vaccine concepts, targets and constructs inspired by new observations or understanding about the nature of the influenza virus or the human response to it and (b) applications of radically new technologies for disease protection, such as production of immunogens using synthetic biology or radical genetic engineering approaches
4. **Enabling advances**: including challenge models to quickly demonstrate safety and proof-of-concept for influenza vaccines

We also would be very happy to receive proposals that describe approaches for employing multiple interventions in combination.

In addition, we may entertain concept proposals related to use of DNA/RNA based delivery of longer acting universal influenza monoclonal antibody for passive prophylaxis or use of such monoclonals for exploring appropriate epitopes for universal influenza vaccine, if generally aligned with our iTPP.

We will NOT consider funding for:

- Marginal improvements in current seasonal influenza vaccines
- Precedented approaches using biosimilars to antigens or adjuvants currently in clinical development
- Basic studies of pathogen or human biology without a clear component that tests the potential for translation into product
- Development of assays, new reagents, adjuvants, or production technologies improving licensed and late-stage vaccines already in development
- Therapeutic monoclonal antibodies for treatment of influenza patients.

Award

Pilot awards ($250,000 up to $ 2 million):

This request for proposals intends to fund pilot awards of up to USD $2 million over 2 years, with the anticipation that one or more pilot projects, on demonstration of promising proof-of-concept data (e.g., from animal models), may be invited to apply for a full award up to USD $10 million. Full awards would be intended to fund IND-enabling and clinical studies.

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Pilot awards do not include a requirement for an industry or translation partner but such partnerships would still be welcome. Industry is also welcome to apply directly for the pilot award. Successful pilot award recipients will have the opportunity to apply for additional funding, which could include grants, program related investments and/or contracts and must include a biopharmaceutical industry or other translational partner.

We reserve the right to determine eligibility for subsequent funding for this call based on these characteristics.

Suggested Reading

Please note: these suggested readings obviously do not represent an exhaustive list of all universal influenza vaccine activities and concepts and very few, if any, are likely to meet all the requirements of our iTPP (described below). We include these for illustrative purposes only and encourage applicants to understand and think beyond the ideas discussed below.

1. Arun Kumar, *et al*. (2018) Novel Platforms for the Development of a Universal Influenza Vaccine. *Frontiers in Immunology*. (doi:10.3389/fimmu.2018.00600).
2. James E. Crowe, Jr. (2017) Is it Possible to Develop a "Universal" Influenza Virus Vaccine? Potential for a Universal Influenza Vaccine. *Cold Spring Harbor Perspectives in Biology*. (doi:10.1101/cshperspect.a029496).
3. Sarah F. Andrews, *et al*. (2017) Is it Possible to Develop a "Universal" Influenza Virus Vaccine? Immunogenetic Considerations Underlying B-Cell Biology in the Development of a Pan-Subtype Influenza A Vaccine Targeting the Hemagglutinin Stem. *Cold Spring Harbor Perspectives in Biology*. (doi:10.1101/cshperspect.a029413).
4. Florian Krammer, *et al*. (2017) Is it Possible to Develop a "Universal" Influenza Virus Vaccine? Toward a Universal Influenza Virus Vaccine: Potential Target Antigens and Critical Aspects for Vaccine Development. *Cold Spring Harbor Perspectives in Biology*. (doi:10.1101/cshperspect.a028845).
5. Davide Angeletti and Johnathan W. Yewdell. (2017) Is it Possible to Develop a "Universal" Influenza Virus Vaccine? Outflanking Antibody Immunodominance on the Road to Universal Influenza Vaccination. *Cold Spring Harbor Perspectives in Biology*. (doi:10.1101/cshperspect.a028852).
6. Francesco Berlanda Scorza, *et al*. (2016) Universal Influenza Vaccines: Shifting to Better Vaccines. *Vaccine*. (doi:10.1016/j.vaccine.2016.03.085).
7. Erin Sparrow, *et al*. (2016) Passive Immunization for Influenza through Antibody Therapies, a review of the pipeline, challenges and potential applications. *Vaccine*. (doi:10.1016/j.vaccine.2016.08.057).

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Target Product Profile

Indication	Protection from morbidity and mortality caused by all subtypes of circulating and emerging (drifted and shifted) Influenza A subtype viruses and Influenza B lineage viruses
Target Population	From six weeks with no upper age limit. High risk populations including pregnant women, children with underlying medical conditions and the elderly
Efficacy	Protection of at least 70% against moderate to severe disease caused by all strains of influenza A and B across all age groups
Duration of protection	Three to five years
Onset of immunity	Four weeks
Herd protection	Desirable
Dosing schedule	Vaccine naïve subjects (pediatric): No more than 3 doses over a 5 year period, compatible with current EPI schedule including co-administration Influenza primed subjects: Single or two dose primary series, booster every five years Single or two doses in Pregnant women
Vaccine Safety	No worse than EPI vaccines
Stability/ Shelf life	Minimum shelf life of 2 years at 2-8 deg C
Product registration path	Universal flu vaccine is likely to be licensed as Seasonal flu vaccine also useful for pandemic, based on clinical efficacy against circulating influenza strains with passive serum transfer studies in appropriate animal model (ferrets?) showing protection against pandemic strains
WHO PQ date	2027
Primary target delivery channel	Routine immunization for infants/children Pre-pandemic global campaign
Target countries	All

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Rules and Guidelines

Applications due no later than June 22, 2018, 11:30 a.m. U.S. Pacific Daylight Time

Overview

Grand Challenges is a family of initiatives fostering innovation to solve key problems in global health and development for those most in need. Each initiative is an experiment in the use of challenges to focus innovation on making an impact. Individual challenges address some of the same problems, but from differing perspectives.

Grand Challenges principles

1. Strategic and well-articulated Grand Challenges serve both to focus research and development efforts and to capture the imagination of and engage the world's best researchers and innovators. The Grand Challenges model focuses on seeking solutions to well-defined problems. The initiative brings these problems to the attention of relevant communities of solvers, both individuals and organizations, and invites creative and forward-thinking approaches to address issues that, if solved, can dramatically improve the world we live in.
2. Projects are selected based on public and transparent calls for proposals seeking the best ideas. The Grand Challenges programs do not purport to know the solutions to the world's most pressing development issues—but they are willing to take risks and invest to create new solutions. The Grand Challenges model aims to engage new solvers with fresh ideas.
3. Funders, innovators and other stakeholders actively collaborate to accelerate progress and promote advances to ensure they serve those most in need. The public, private, academic and nonprofit sectors must work together to accelerate and scale up innovations that can improve the lives of those most in need.
4. Projects are selected not only for scientific excellence but also for the likelihood that they will achieve the desired scale and impact. Successful applicants present projects that, when proven successful through the collection of rigorous evidence, have the potential to serve those most in need. Investing in scientific innovation—as well as in the business and social innovation needed to increase impact at scale—will help ensure that these efforts have the greatest possible impact in terms of lives saved or improved.
5. Researchers and innovators work to ensure that the fruits of their projects are accessible and available to those most in need. Fostering ties to industry, both by helping bridge the private and public sectors or by directly funding a company, can create sustainable enterprises, and reduce the time from discovery to development, production and impact. Key to this is developing global access strategies to ensure that those most in need benefit from new solutions. For additional information on the Foundation's global access and open access requirements, please see the Foundation's Global Access website and Open Access Policy.

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Application Instructions

Key dates and deadlines for this Grand Challenges

Key Dates*	Event
April 27, 2018	Topic Published
June 22, 2018, 11:30am PDT	Letter of Inquiry due

*Note, any changes to these dates will be posted on the Grand Challenges website

Applicants can expect to hear if their LOI is selected to move forward in August 2018. Full proposals will be invited from selected applicants and we expect to make funding decisions by November 2018.

How to Apply

Changes to the Rules and Guidelines and to the Frequently Asked Questions (FAQ) document will be posted periodically on the webpage for each open challenge at the Grand Challenges website (gcgh.grandchallenges.org), including any changes to the dates listed above. Please read the current FAQ before submitting any questions or concerns.

Letter of Inquiry (LOI) Application Format

We encourage you to use the provided RFP-specific LOI instructions document, which can be downloaded after clicking "Apply Now" on each application page and can also be found in the Supporting Material. You are required to submit either a Microsoft Word® or PDF document; no more than four pages in length (including references). Please do not include a cover sheet with your proposal. A cover sheet will be automatically generated from your registration data.

The LOI application must include the following sections and must not exceed the **total page limit of 3 pages**. An outline for the LOI application is provided below:

1. **Goals and Rationale**
   Describe the specific goals of the project and how they are responsive to the objectives noted in the Request for Proposals. Describe the advance in universal influenza vaccine development that the proposed project will deliver. Specify any hypotheses that will be tested. If applicable, briefly present any relevant preliminary data. Indicate the expected results from the project. Briefly indicate what the next steps might be and how the project's results could lead to successful development of a universal influenza vaccine candidate. At the beginning of this section, include one or two sentences in bold that capture the essence of your idea.

2. **Study Design, Scope, and Approach**
   This section should include a clear description of the research questions, the proposed research study, and how your project differs from current approaches. Please include your study design, including key milestones, as well as any supporting services (e.g., common assays, animal models, etc.) you may need to complete your work.

3. **Organization and Investigator Capabilities & Collaboration**
   Summary of the lead PI's and applicant organization's previous experience and expertise that is relevant to this call. A description of collaborations, and if/how partnerships will be established (letters of support are not expected at this stage). This should include a description of where samples will be stored and analyzed, a plan to store isolates for future studies as well as a plan to share data.

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4. **Budget**
   Summarize by research objective for each year of the project; **we will consider proposals for pilot awards of up to USD $2 million over two (2) years.**

Proposals for this Grand Challenge must be submitted prior to the deadline of 11:30am Pacific Daylight Time on June 22, 2018.

Tips for Applicants

- Your proposal must comply with all restrictions and guidelines for the challenge to which you are applying, and it must explain how it addresses a key need highlighted in the challenge description. In addition, the challenge description highlights ideas that will not be funded, so applicants should make sure that their idea does not fall into one of these categories. The foundation only funds projects responsive to the Request for Proposals as it has a number of other avenues of funding for the equally important research that is otherwise within currently accepted program paradigms.
- The work proposed in your application must include a clear set of key experiments or activities that test your idea in a way that could provide sufficient evidence to warrant funding. Proposals with vague descriptions or vague testing methodologies will not be funded.
- The proposed project should have a clearly articulated goal, usually in terms of proving a hypothesis that if proved could lead to significant impact if properly scaled. The project plan should be organized by objectives that are logically required to achieve the goal of the project and should include a plan to **prove or disprove the underlying hypothesis** in an efficient and logical manner.
- Not all the reviewers for each proposal will be deep experts in the field. To maximize the chance of being funded, proposals should be written in clear language without jargon specific to a particular field.
- Your organization must meet the eligibility requirements as specified in this document and further refined in each of the challenge calls.

During the application process you will be required to confirm that you have read and understand the Website Terms of Use and Privacy Policy, and these Rules and Guidelines, and acknowledge that any information that you provide to us (either orally or in writing) will be subject to and handled in accordance with such provisions.

Eligibility Criteria

Grand Challenges is open to both foreign and domestic organizations, including non-profit organizations, for- profit companies, international organizations, government agencies and academic institutions. Individuals and organizations classified as individuals for U.S. tax purposes are not eligible to receive an award from the foundation as part of the Grand Challenges initiative.

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Upon registration, applicants must provide information about the tax status of their organization as different terms and conditions may apply. You should confirm your organization's tax status with the appropriate advisor or entity within your organization such as your grants or contracts department, finance, or office of sponsored research. The foundation may request additional information regarding your tax status. For information about tax statuses, you may check with your own advisors and review information provided on the Internal Revenue Service web site at: www.irs.gov.

Each challenge published under the Grand Challenges may include additional instructions with regard to specific limitations regarding eligibility for that challenge.

Review Process

The review of proposals is a critical element of the Grand Challenges initiative - its goal is to filter and harness creative ideas. For each challenge call, we advertise a set of challenges carefully defined to elicit innovative responses to critical barriers in global health and development.

Handling of Proposals

The foundation has put in place policies and procedures, exclusive to the Grand Challenges initiative, intended to restrict public dissemination of application materials. These policies and procedures include, when possible, having external reviewers sign confidentiality agreements and requiring that reviewers destroy or return to the foundation all copies of information acquired or created during the course of performing a review. In some instances, however, we are unable to put in place confidentiality agreements or to police the use of application materials.

Additionally, the foundation is required by the IRS to publish a list of its grants. The foundation also provides general descriptions of its grants on its web sites, in press releases, and in other marketing materials. Subject to this Privacy Policy, the foundation may also share information you provide to us (either orally or in writing) with third parties, including external reviewers, consultants, contingent workers, key partners and co-funders. These Rules and Guidelines are subject to these Terms of Use.

Review of Proposals

Due to the large number of proposals anticipated, applicants with proposals that are not selected for award will receive a notification of decline without specific feedback. Nonetheless, applicants are encouraged to submit ideas in future years.

The Grand Challenges application review process for Phase I is executed in four steps:

1. The first step consists of screening LOI applications to evaluate whether proposals address the key needs described in the topic. We screen for responses that are completely unrelated or specifically excluded in the topic description. Excluded responses encompass ideas related to the topic but that, for strategic reasons, we are not funding under the Grand Challenges initiative. In addition, we exclude proposals considered incremental advances, appropriate responses that are similar to work in which the foundation or other Grand Challenges programs are already investing, or avenues of inquiry we deem ill-suited to the Grand Challenges initiative. Applicants with proposals removed from consideration during the screening process will be notified that their proposals were declined but will not receive specific feedback.
2. In the second step, reviews are chaired or co-chaired by technical leads from the foundation and other funding partners and are conducted by reviewers both outside and within the foundation. Reviewers are selected from the world's leading researchers and comprise both experts in the

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topic area and experts in complementary areas. Not all reviewers have deep expertise in the topic; please consider this information when drafting your proposal. Unlike most scientific funding programs, reviewers are encouraged to champion specific projects to be funded as part of the portfolio, without the need for a broad consensus on selections.

In order of importance, the criteria considered in these reviews are:

- Potential to lead to solutions with substantial **impact** related to the specific topic
- **Scientific and technical excellence & innovation**, including:
  - creativity of the project's approach and clear differentiation from existing approaches
  - a clear and rigorous conceptual framework for the activities
- Project Plan, including:
  - investigator and organization capabilities and potential for collaboration
  - **value** in terms of appropriateness of the budget and timeline relative to project complexity, risk, and potential impact

3. The third step is the validation and final selection of the LOI applications to be invited to submit full proposals by an Executive Committee. Invited proposals will be provided additional technical instructions at that time.

4. The fourth step involves repeating step two, but with regard to evaluation of the full proposal and with the inclusion of a more detailed review of the project plan and capability to execute the work.

5. The fifth step is the validation and final selection of the proposals to be funded by the Executive Committee. The Executive Committee may recommend the proposal be funded, subject to specific modifications which will be negotiated as part of the award process.

6. The sixth and final step is a due diligence review and to directly discuss and negotiate any adjustments to the proposed project recommended by the Executive Committee. Investigators will be contacted as part of the due diligence review.

Management of Conflict of Interest

To identify and avoid conflicts of interest among application reviewers, such reviewers will not be permitted to review proposals from organizations with which the reviewer has self-identified conflicts of interest.

Eligibility and Notifications

A. Allowable Costs

Grant funds may be used for the following costs: personnel, necessary travel, supplies, contracted services, sub-grants, and consultants. Partial or full support for equipment may be requested subject to the circumstances described below. Please provide budget estimates according to these categories. The foundation provides a limited amount of indirect costs based on the nature of the applicant organization.

B. Privacy Notice

To help foundation staff in their evaluation and analysis of projects, all documents, communications and associated materials submitted to the foundation (collectively, "Submission Materials") will become the property of the foundation and may be subject to external review by independent subject matter experts in addition to analysis by foundation employees, contingent workers and/or consultants. Please consider carefully the information included in the Submission

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Materials. If you have any doubt about whether to disclose confidential or proprietary information, the foundation recommends you consult with your legal counsel. You may wish to consider whether such information is critical for evaluating the submission, and whether more general, non-confidential information may be adequate as an alternative for these purposes. We respect confidential information we receive. Nonetheless, notwithstanding your characterization of any information as being confidential, the foundation may disclose all information contained in Submission Materials to the extent it determines is necessary to evaluate them and the manner and scope of potential funding and as may be required by law.

C. Disclaimer

Neither these Rules & Guidelines nor the associated challenge calls constitute an offer to contract or award grant funds. The foundation assumes no responsibility for the applicants' cost to respond to this these calls.

D. Release and Verification

In exchange for the opportunity to be considered for a grant, contract or program-related investment (PRI) (per the relevant challenge call), the applicant agrees that the foundation may, in its sole discretion: (1) amend or cancel any challenge calls, in whole or in part, at any time; (2) extend the deadline for submitting responses; (3) determine whether a response does or does not substantially comply with the requirements of the challenge call and/or these Rules & Guidelines; (4) waive any minor irregularity, informality or nonconformance with the provisions or procedures of the challenge call and/or these Rules & Guidelines; (5) issue multiple awards; (6) share responses generated by the challenge call and/or these Rules & Guidelines with foundation staff, consultants, contingent workers, subject matter experts, and potential co-funders; and (7) copy the responses.

Applicant agrees not to bring a legal challenge of any kind against the foundation relating to the foundation's selection and award of any grant, contract or award arising from these Rules & Guidelines and the associated challenge calls.

E. Warranty

By providing any Submission Materials, the sender(s) and applicant warrant to the foundation that they have the right to provide the information submitted and that such information is accurate. Any material misrepresentation, including omissions, may disqualify Submission Materials from consideration for a grant, contract or PRI award. Applicants with questions concerning the contents of their Submission Materials may contact the foundation at: grandchallenges@gatesfoundation.org.

F. Intellectual Property (IP)

Since the output of this program may lead to innovative technologies, techniques and/or products that will result in improved health and development-related products for those in greatest need in the developing world, the successful development of these high priority products may require substantial involvement and support of private sector industries as sub-contractors, and may also involve collaborations with multiple organizations, including academic and/or non-profit research institutions.

It is the intent of this program to support the formation of appropriate public-private partnerships that are essential to meet urgent global health and global development needs. IP rights and the management of IP rights are likely to play an important role in achieving the goals of this program. To this end, the foundation requires that, even at the LOI application stage, all applicants seriously consider their willingness to submit a full proposal in compliance with the foundation's proposal

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guidelines, a portion of which asks for certain information and intentions regarding intellectual property and global access concerns. Specifically, the foundation requires that you agree to use good faith efforts to conduct and manage the research, technologies, information and innovations involved in the Project in a manner that enables (a) the knowledge and information gained during the Project to be promptly and broadly disseminated, and (b) the intended product(s) to be made available and accessible at an affordable price to people most in need in the developing countries of the world. The foundation refers to this as "Global Access."

As part of the foundation's review and evaluation of each full proposal, due diligence will be conducted with respect to each participant's ability and commitment to manage intellectual property in a manner consistent with the stated scientific and charitable goals of the foundation. Due diligence activities may include inquiry into an applicant's: (1) Freedom to operate (FTO) and ability to freely use and acquire needed background technology; and (2) Commitment to promote the utilization, commercialization and availability of inventions for public benefit in, or the benefit of, developing countries. In order to facilitate this due diligence process applicants are encouraged to provide information with respect to the items above in their Submission Materials.

Applicants are also expected to make new information and materials known to the research and/or medical communities in a timely manner through publications, web announcements, progress reports to the foundation, and other appropriate mechanisms. These concepts may be discussed at some length with the applicants invited to submit full proposals, and will be addressed (to the extent appropriate) within each final grant agreement. The Global Access Strategy, developed in connection with each grant awarded to each applicant who submits a successful proposal, will also include provisions defining these concepts.

G. Compliance with Laws and Other Requirements

1. **Compliance with Laws**. In carrying out the individual project, you will comply with all applicable laws, regulations, and rules and will not infringe, misappropriate, or violate the intellectual property, privacy, or publicity rights of any third party.

2. **Compliance with Requirements**. You will conduct, control, manage, and monitor the individual project in compliance with all applicable ethical, legal, regulatory, and safety requirements, including applicable international, national, local, and institutional standards ("Requirements"). You will obtain and maintain all necessary approvals, consents, and reviews before conducting the applicable activity. As a part of your annual progress report to the foundation, you must report whether the individual project activities were conducted in compliance with all Requirements.

   If the project involves:

   (a) any protected information (including personally identifiable, protected health, or third-party confidential), You will not disclose this information to the foundation without obtaining the foundation's prior written approval and all necessary consents to disclose such information;

   (b) children or vulnerable subjects, you will obtain any necessary consents and approvals unique to these subjects; and/or

   (c) any trial involving human subjects, you will adhere to current Good Clinical Practice as defined by the International Council on Harmonisation (ICH) E-6 Standards (or local regulations if more stringent) and will obtain applicable trial insurance.

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Any activities by the foundation in reviewing documents and providing input or funding does not modify your responsibility for determining and complying with all Requirements for the project.

3. **Indemnification**. If the project involves clinical trials, trials involving human subjects, post-approval studies, field trials involving genetically modified organisms, experimental medicine, or the provision of medical/health services ("Indemnified Activities"), you will indemnify, defend, and hold harmless the foundation and its trustees, employees, and agents ("Indemnified Parties") from and against any and all demands, claims, actions, suits, losses, damages (including property damage, bodily injury, and wrongful death), arbitration and legal proceedings, judgments, settlements, or costs or expenses (including reasonable attorneys' fees and expenses) (collectively, "Claims") arising out of or relating to the acts or omissions, actual or alleged, of you or your employees, subgrantees, subcontractors, contingent workers, agents, and affiliates with respect to the Indemnified Activities. You agree that any activities by the foundation in connection with the project, such as its review or proposal of suggested modifications to the project, will not modify or waive the foundation's rights under this paragraph. An Indemnified Party may, at its own expense, employ separate counsel to monitor and participate in the defense of any Claim. Your indemnification obligations are limited to the extent permitted or precluded under applicable federal, state or local laws, including federal or state tort claims acts, the Federal Anti-Deficiency Act, state governmental immunity acts, or state constitutions. Nothing in this agreement will constitute an express or implied waiver of your governmental and sovereign immunities, if any.
4. **Insurance**. You will maintain insurance coverage sufficient to cover the activities, risks, and potential omissions of the project in accordance with generally-accepted industry standards and as required by law. You will ensure your subgrantees and subcontractors maintain insurance coverage consistent with this section.

Privacy Notice and Terms of Use

Our full privacy policy and terms of use are located at Privacy and Terms of Use.

Representation: By providing any Submission Materials, the sender represents to the Bill & Melinda Gates Foundation that he/she (i) has the authority to bind the named Applicant to the terms set forth above, without amendment, (ii) agrees to be bound by such terms; and (iii) has the right to provide the information submitted.

Please direct all questions about this initiative to: grandchallenges@gatesfoundation.org.

Frequently Asked Questions

Answers to many application questions can be found on the Frequently Asked Questions document posted on the webpage for each open challenge at the Grand Challenges website (gcgh.grandchallenges.org).

Inquiries

Please direct all questions about this initiative, selection criteria or application instructions by e-mail to the following address: grandchallenges@gatesfoundation.org.

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Bruce Gellin, MD, MPH
President, Global Immunization
Sabin Vaccine Institute

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From: Marston, Hilary (NIH/NIAID) [E]
Sent: Thu, 26 Apr 2018 21:10:30 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Fwd: AMP slides
Attachments: Clinical MAb slides for IAVI mtg v3.pptx, ATT00001.htm

Begin forwarded message:

From: "Ledgerwood, Julie (NIH/NIAID) [E]" (b)(6)
Date: April 16, 2018 at 2:48:20 PM EDT
To: "Marston, Hilary (NIH/NIAID) [E]" (b)(6) "Sitar, Sandra (NIH/VRC) [C]" (b)(6)
Subject: RE: AMP slides

I have attached my slides from Friday---feel free to use any.

From: Marston, Hilary (NIH/NIAID) [E]
Sent: Monday, April 16, 2018 2:45 PM
To: Sitar, Sandra (NIH/VRC) [C] (b)(6) Ledgerwood, Julie (NIH/NIAID) [E] (b)(6)
Subject: RE: AMP slides

Thanks so much! Do you also have the 30mg/kg to 10 mg/kg graph?

From: Sitar, Sandra (NIH/VRC) [C]
Sent: Monday, April 16, 2018 2:44 PM
To: Ledgerwood, Julie (NIH/NIAID) [E] (b)(6) Marston, Hilary (NIH/NIAID) [E] (b)(6)
Subject: Re: AMP slides

Hi Hilary,

Attached is the AMP intro and map slide that we have been using (courtesy of HVTN). For assistance in accuracy with map making, I'm also including the site memos from the HVTN team.

Please let me know if anything additional would be useful.

Best,
Sandra

From: Julie Ledgerwood (b)(6)
Date: Monday, April 16, 2018 at 7:24 PM

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To: "Marston, Hilary (NIH/NIAID) [E]" (b)(6)
Cc: Sandra Sitar (b)(6)
Subject: RE: AMP slides

Hi Hilary
Yes—of course. Sandra will send you all that we have.
JL

From: Marston, Hilary (NIH/NIAID) [E]
Sent: Monday, April 16, 2018 1:17 PM
To: Ledgerwood, Julie (NIH/NIAID) [E] (b)(6)
Subject: AMP slides

Hey Julie – Would you mind sharing some of your AMP slides? I wanted to weddle-ize the map at least (with attribution of course). Will send you the weddle versions.

Hilary Marston, MD, MPH
Medical Officer and Policy Advisor for Global Health
NIAID Office of the Director
(b)(6)
(b)(6) (cell)

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National Institute of Allergy and Infectious Diseases
Dale and Betty Bumpers Vaccine Research Center

VRC HIV MAbs in Clinical Trials

Julie E. Ledgerwood, D.O.ChiefClinical Trials Program Vaccine Research CenterNational Institute of Allergy and Infectious DiseasesNational Institutes of Health

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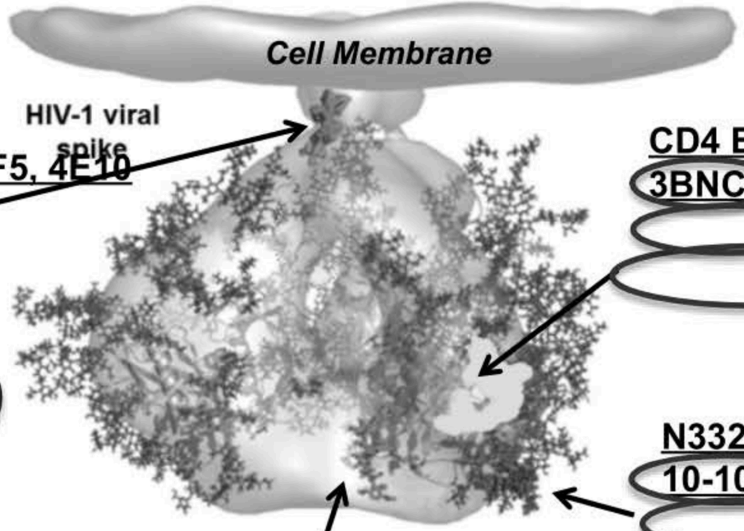
HIV-1 Neutralizing mAb Products in Clinical Development

4 major sitesin 2018



gp41 MPER:2F5, 4E10

10e8v5R

Bi-specific (10e8/anti-CD4)

Tri-specific (CD4bs, V2, MPER)

eCD4-Ig (CD4, anti-CCR5)

CD4 Binding Site:VRC01, VRC01-LS, CH31
3BNC117, 12A12, CH103, VRC07-523LS, Z

N332 Glycan Supersite:PGT121, PGT128
10-1074

V1V2 Glycan:PG6, PG16, CH01-04
PGT141-45, PGDM1400
CAP256-VRC26

Phase 1

GMP manufacturing

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Cryo-EM of viral spike by Subramaniam group. Fit with atomic level structures from Kwong and Wilson groups

se 1/1b Trials Summary(VRC 601, VRC 602

Sep 24, 2015

Clinical & Experimental Immunology

Safety, Pharmacokinetics and Neutralization of the Broadly Neutralizing HIV-1 Human Monoclonal Antibody VRC01 in Healthy adults.Ledgerwood JE, Coates EE, et al., Clin Exp Immunol. 2015

Dec 23, 2015

Science Translational Medicine

Virologic effects of broadly neutralizing antibody VRC01 administration during chronic HIV-1 infection.Lynch RM, Boritz E, Coates EE, et al, Sci Transl Med. 2015 Dec 23;7(319):319ra206.

Nov 14, 2017

PLOS | MEDICINE

Safety, pharmacokinetics, and immunological activities of multiple intravenous or subcutaneous doses of an anti-HIV monoclonal antibody, VRC01, administered to HIV-uninfected adults: Results of a phase 1 randomized trial.Mayer et al. PLoS Med. 2017 Nov 14;14(11):e1002435.

- VRC01 safe Serum concentrations of >20 mcg/mL maintained for 8 weeks at 20 mg/kg doseNo anti-drug antibody VRC01 half life: 15 daysVRC01 reduced VL by >1 Log in 6/8 viremic subjectsVRC01 Advanced to Phase 2b efficacy testing in AMP

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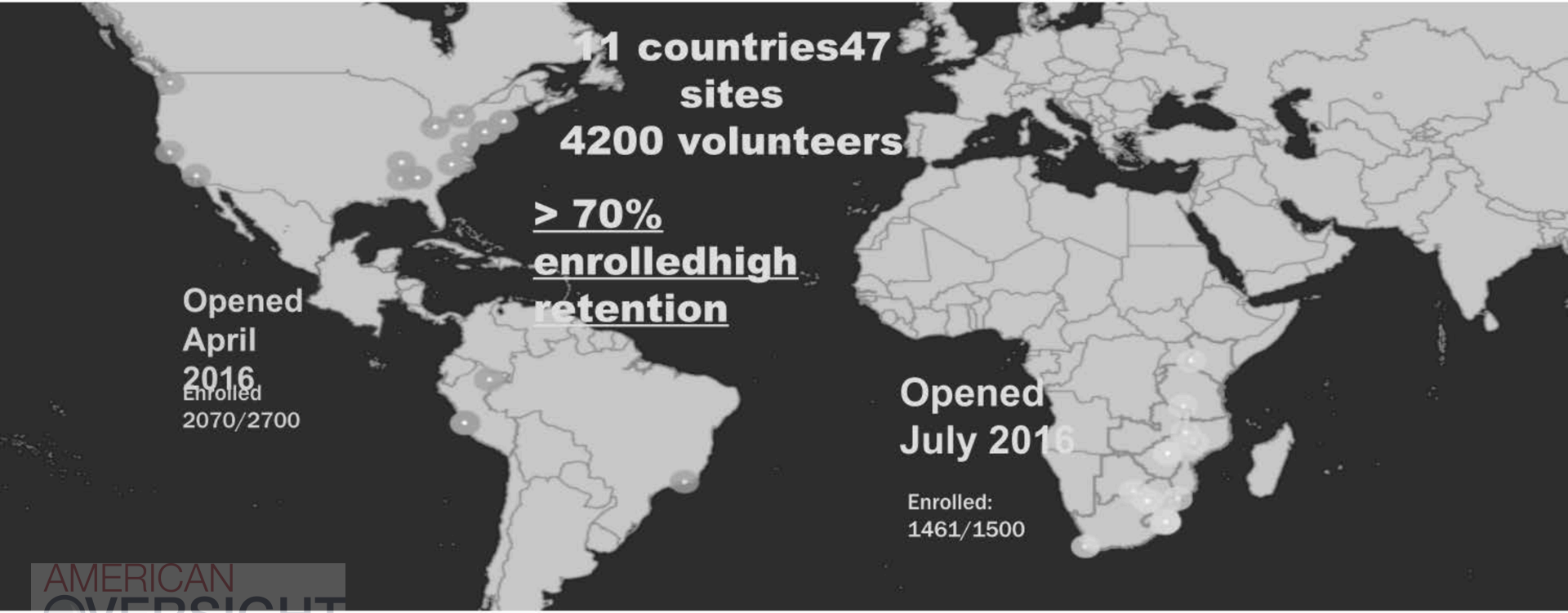
Antibody-Mediated Prevention (AMP)

VRC01 mAb phase 2b studies in high risk men and women



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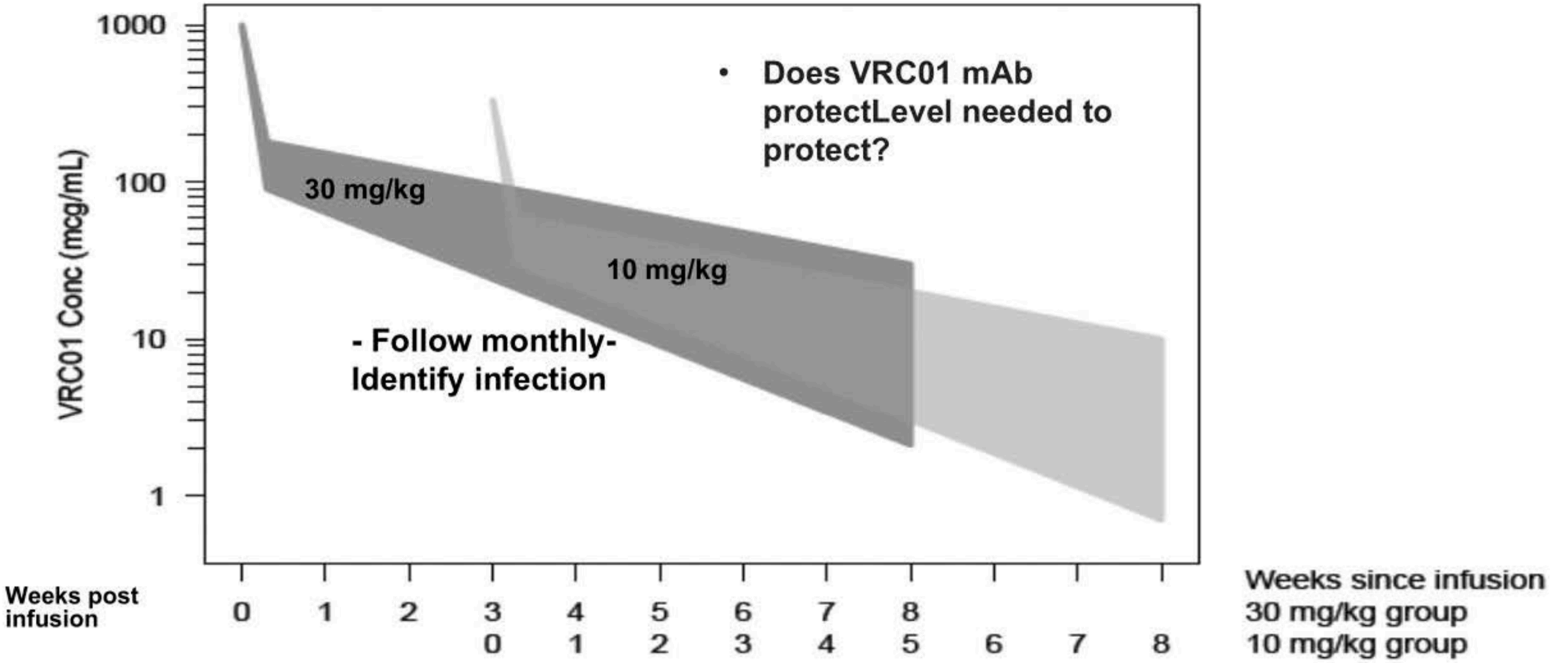
DAIDS

Associate Serum mAb Level with Time of Infection



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- VRC01 administered at 30 mg/kg, or 10 mg/kg, vs placeboAdministered every 8 weeks by IV infusion

What Happens With Success:
i.e. If this antibody decreases risk of infection

- Learn how much antibody (serum level) is needed to protectLearn if viral resistance is important

- IF AN ANTIBODY WORKSIncentive to fully develop the next generation mAbs: more potent and long actingIncentive for commercial development of HIV mAbsImplications for vaccine development

SQ route once every 3-4 months

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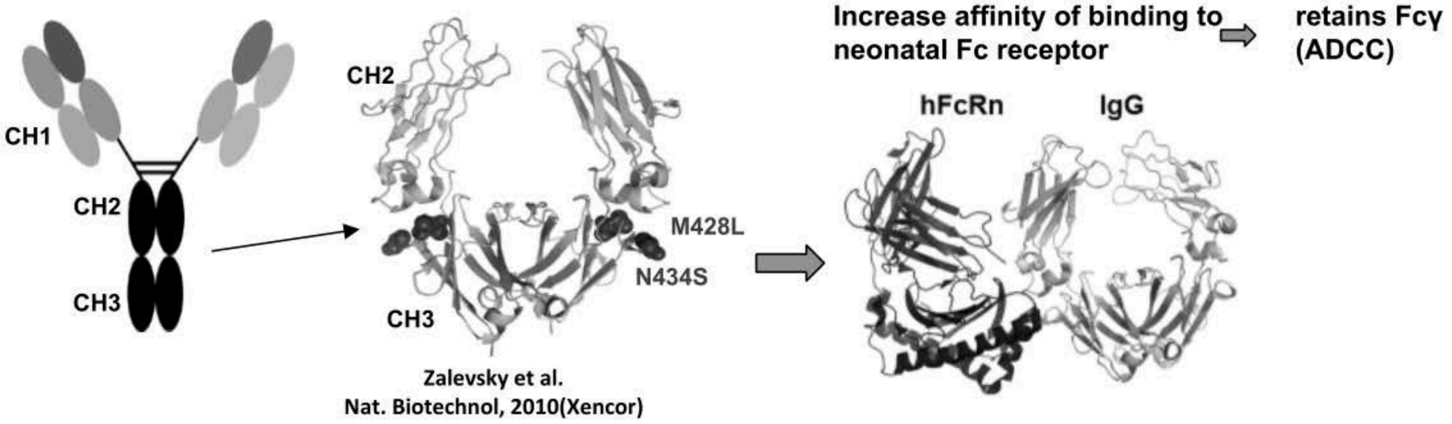
n – increased affinity for FcRn:Prolongs circulat



- Fc region binds with high affinity to FcRn at acidic pH (<6.5) in endosomeProtects antibody from endosomal degradation IgG released back into circulation at physiological pH (7.4)Results in prolonged circulating half life

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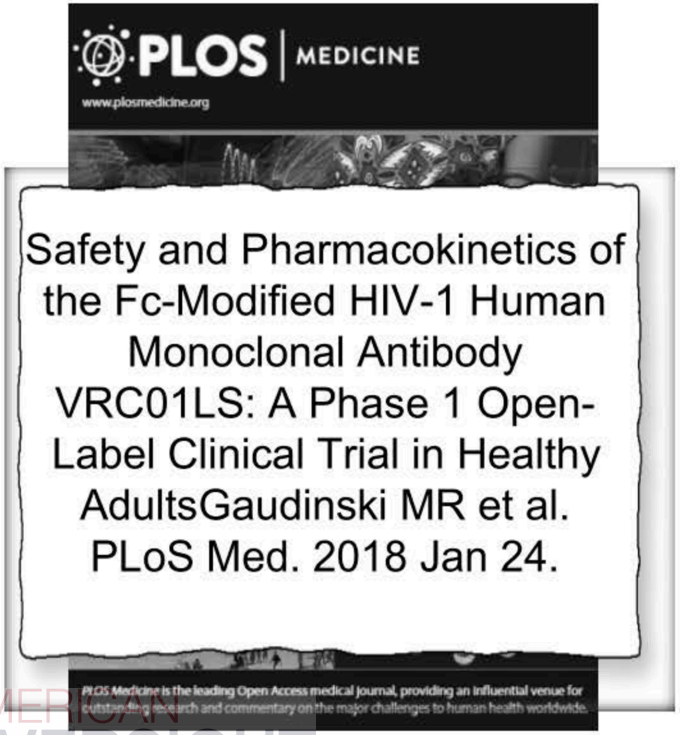
VRC01LS Phase 1 Trial Summary
(VRC 606)



- VRC01LS safe and well-tolerated when delivered IV or SCNo serious adverse events or dose-limiting toxicitiesVRC01LS retained its expected neutralizing activity in serum and anti-VRC01 antibody responses were not detected T ½ is 71 days (>4 fold longer than VRC01)

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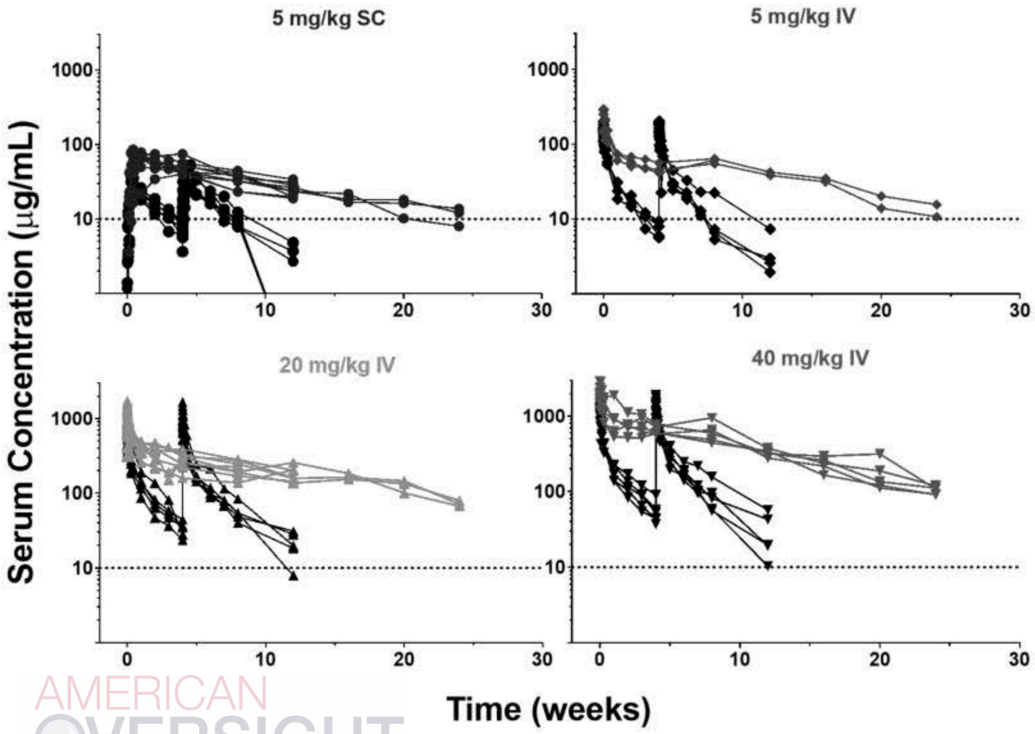
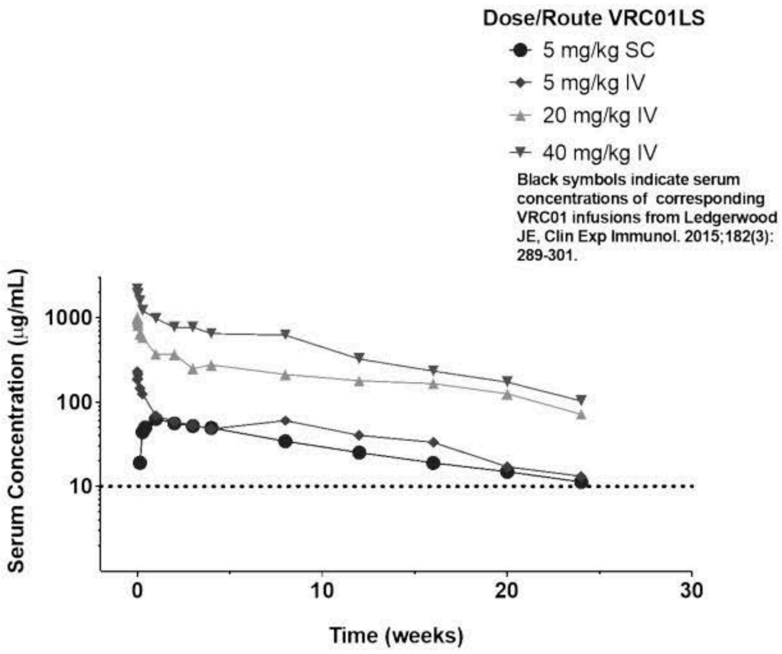
Serum Antibody Concentration (mcg/ml) of VRC01 and VRC01LS Following Single Administrations of VRC01LS





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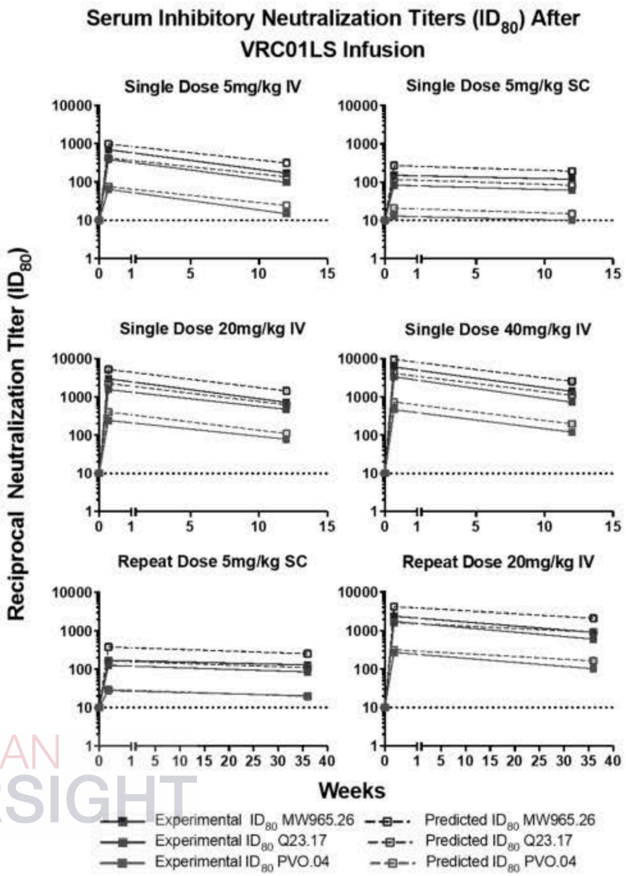
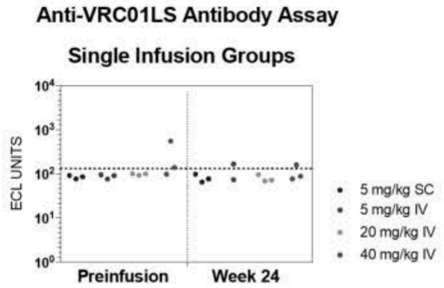
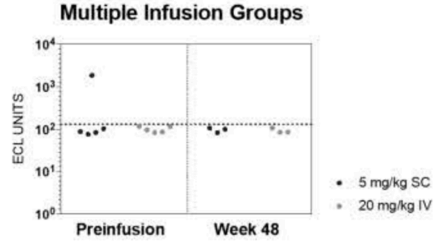
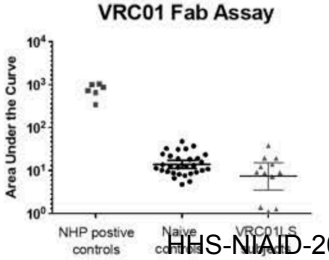
VRC01LS Post Infusion Serum Neutralization was Retained and no Antidrug Antibody (ADA) was Detected









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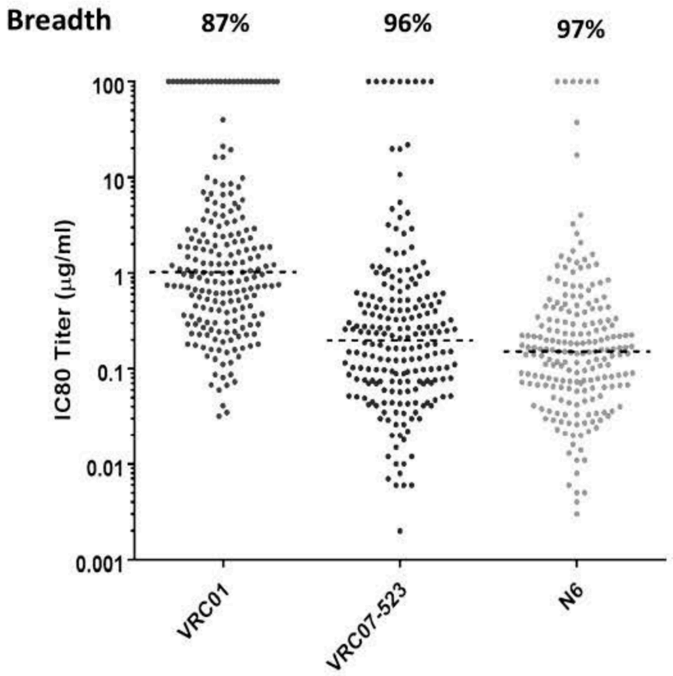
Next Gen Products:Improved Neutralization(VRC07523 and N258.N6)



- 10 fold more potent than VRC01Coverage improves to > 95%,VRC07523LS Phase 1 enrolledN6LS Phase 1 Spring 2018

Panel of 206 Env-pseudoviruses: Doria-Rose, Louder, McKee, Bailer et al.

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VRC07523LS Phase 1 Fully Enrolled

(VRC 605)

- Phase 1, Dose-Escalation, Safety and PKVRC07-523LS Administered IV or SC to Healthy AdultsAdministration (dose/route): Single dose of 1 mg/kg, 5 mg/kg, 20 mg/kg, 40 mg/kg IV, or 5 mg/kg SCRepeat doses of 20 mg/kg IV, or 5 mg/kg SC, at 12 week intervals

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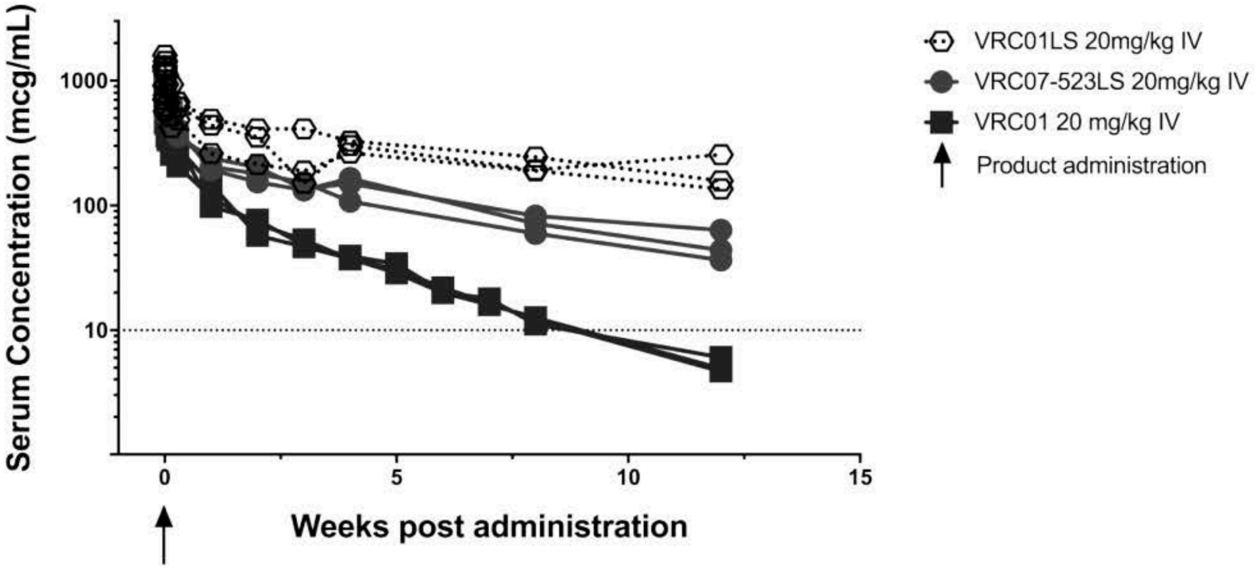
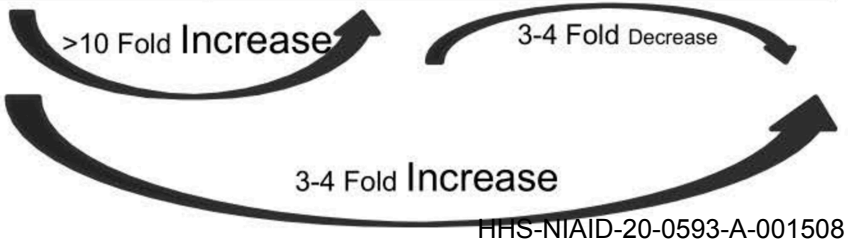
Subject serum concentration (mcg/mL) after a single administration of VRC07-523LS, VRC01LS, or VRC01



AMERICAN OVERSIGHT

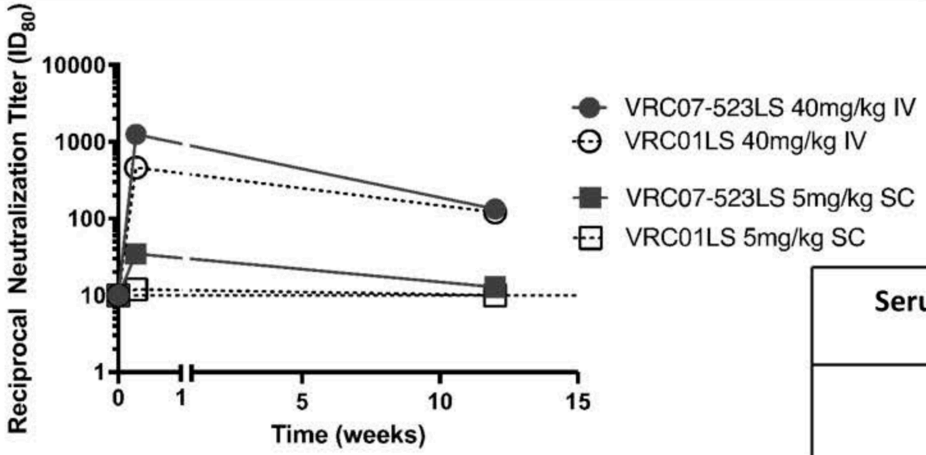
Serum Concentration by Dose 12 Weeks After Single Administration of VRC01, VRC01LS, and VRC07523LS

Serum Antibody Concentrations (mcg/mL)Group arithmetic mean (SD)			
Dose/Route(Single administration: week 0)	VRC01	VRC01LS	VRC07523LS
5 mg/kg IV	3.0 (2.7)	40 (2.7)	12 (1.8)
5 mg/kg SC	2.3 (2.1)	25 (1.7)	6.7 (1.4)
20 mg/kg IV	15 (11)	183 (64)	45 (12)
40 mg/kg IV	30 (19)	326 (35)	85 (30)



AMERICAN OVERSIGHT

Neutralization (ID_{80}) of virus PVO.04 (clade B) for VRC07-523LS compared to VRC01LS



Serum concentration and potency against PVO.04 of VRC07-523LS and VRC01LS at 12 weeks post administration				
	Serum Concentration mcg/mL (SD)		Neutralization titer ID80 (SD)	
Antibody	5mg/kg SC	40mg/kg IV	5mg/kg SC	40mg/kg IV
VRC07-523LS	6.7 (1.4), n=7	85 (30), n=3	13 (5.0), n=3	133 (61), n=3
VRC01LS	26 (7.8), n=18	326 (35), n=5	10 (0), n=3	120 (20), n=5
Fold difference (VRC07-523LS/VRC01LS)	0.25	0.26	1.29	1.11

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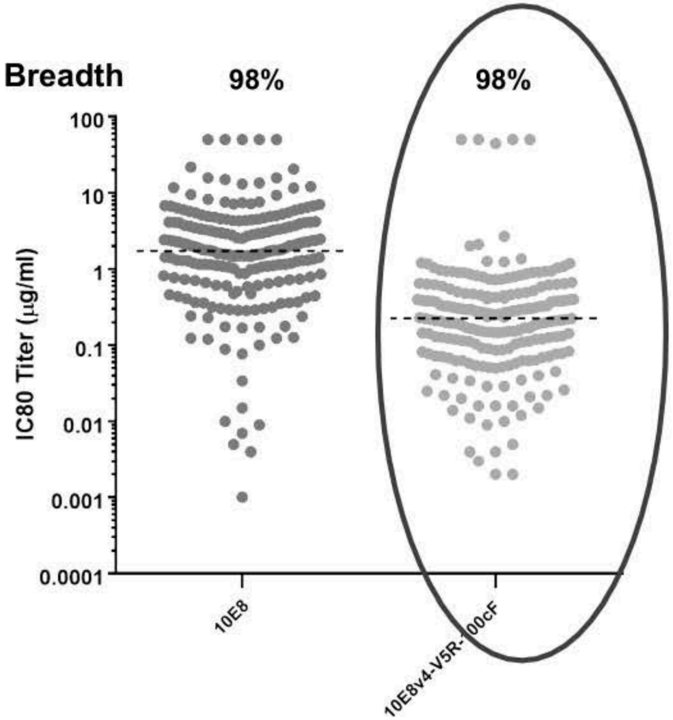
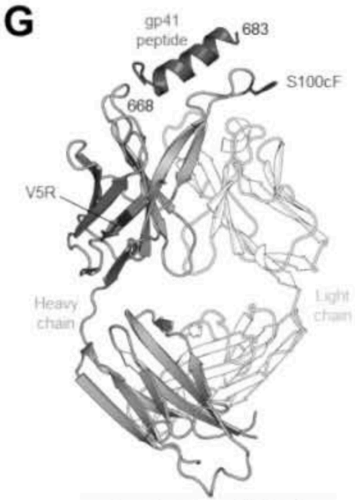
Improved Neutralization of 10e8 variantMPER mAb Clinical Product





Crystal structure of 10E8v4-5R+100cF with gp41 peptide

Engineered: 10e8v5R:10-fold more potent than parental 10e8(Kwong et al, submitted)Added LS mutation=10e8VLSProduct is manufacturedPhase I in mid 2018

Multiclade Virus Panel (n=208) Doria-Rose, Louder, Bailer et al (VRC)

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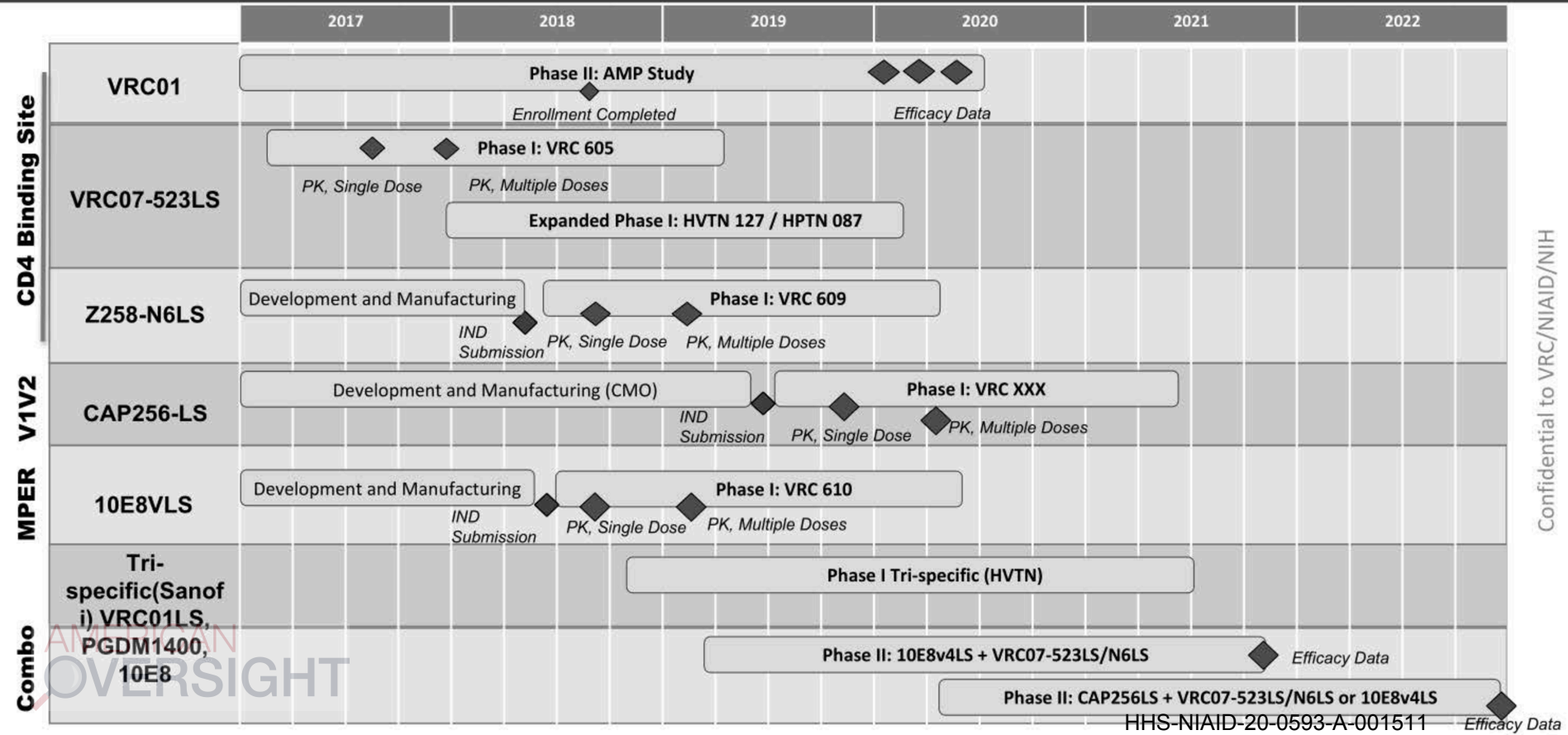
VRC HIV mAb Portfolio



Confidential to VRC/NIAID/NIH

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Summary

- VRC01 AMP efficacy trial will complete enrollment in 2018, data in 2019VRC01LS improves durability compared to VRC01 (>8-fold)VRC07523LS improves durability, neutralization and breadth over VRC0110E8VLS and N6LS will enter Phase 1 testing in mid 2018Combination studies to enter Phase 1/1b testing in 2018

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Acknowledgements

VRC Clinical Trials Program
Preeti Apte Alison BeckNancy Rebecca LampleyBrenda BerkowitzEugeania BurchLarkin Lam LeFloreliz Burgos FlorezCristina MendozaLaura NovikMark CarterGrace ChenEmily O'Callahan Abidemi CoatesPam CostnerJosephine Olalis PittmanSarah PlummerRo CoxJennifer CunninghamAlyssa RothwellJamie SaundersEllie EshunMartin GaudinskiIngelisa SebaSandra SitarStephanie GordonCarmencita TaylorCora Trelles Cartagena GravesMercy GuechCynthia Olga Starr Hendel Somia TrofymenkoOlga HickmanRenunda HicksLyudmyla VasilenkoXiaolin WangWil HolmanKate Houser WhalenPernell Williams Galina Yamshchikov

VRC John MascolaBarney GrahamRick KoupPeter KwongRobert BailerDanny DouekNicole Doria-RoseAmarendra PeguSandeep NarpalaMark LouderSijy O'DellRebecca Lynch Eli BoritzAdrian McDermottDavid CarltonAbe MittelmanMarybeth DaucherLucio GamaJason GallGary NabelRebecca Ruckly

VRC Clinical/RegulatoryCarrie LaurencotMichelle Conan-Cibotti Flo KaltovichJudy SteinVRC Product DevelopmentFrank ArnoldRichard Schwartz David LindsayGretchen ScheiberNIAID, BiostatisticsZonghui HuPK AnalysisEdmund CapparelliCAVD, VIMCM, SeamanD. Montefiori

HVTN, HPTNHVTN 104 and AMP study teams
Mike Cohen
Larry CoreyShelly KarunaKen MayerNIAIDCarl DieffenbachMary Marovich
Sarah ReadSheryl ZwerskiDiana FinziRandy TresslerMary AllenMarga GomezStephen MiguelesMark Connors

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National Institute of Allergy and Infectious Diseases
Vaccine Research Center

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Extra slides

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Potency

Reference: IC_{80} values for VRC01, VRC01LS, and VRC07523LS

Virus strain	Clade	VRC07-523LS	VRC01LS	VRC01	VRC07-523LS vs VRC01fold higher potency
CNE59	AE	0.477	3.14	3.86	8.1
DU156.12	C	0.116	0.392	0.482	4.2
MW965.26	C	0.048	0.149	0.134	2.8
PVO.04	B	0.460	1.431	1.666	3.6
Q23.17	A	0.170	0.209	0.237	1.4
THRO.18	B	6.93	21.4	20.1	2.9
ZM53.12	C	1.17	4.09	5.06	4.3
6322.V4.C1	C	0.135	>50.0	>50.0	>370
CAP210	C	8.63	>50.0	>50.0	>5.8
MuLV	-	>50.0	>50.0	>50.0	NA

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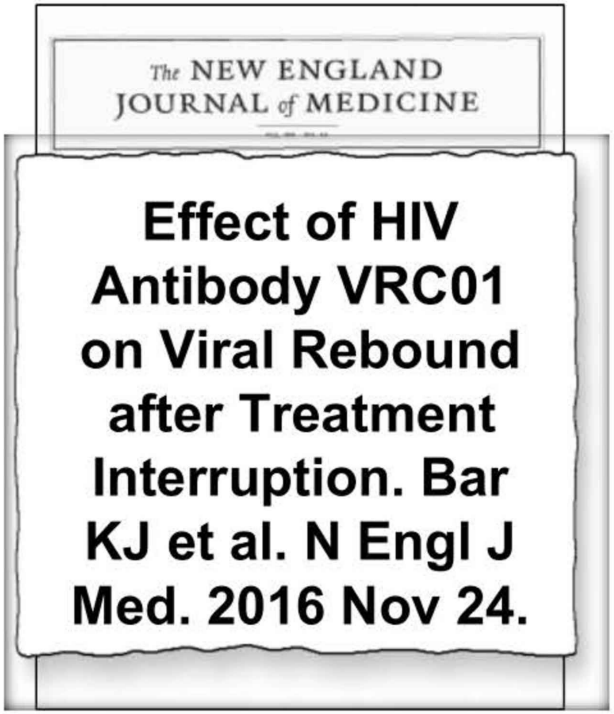
VRC01 Assessed in HIV ATI Clinical Trials

- VRC01 was safe and slightly delayed plasma viral rebound in 2 small ATI trialsVRC01 monotherapy did not maintain viral suppression by week 8 and combination therapy will be needed for a treatment approach



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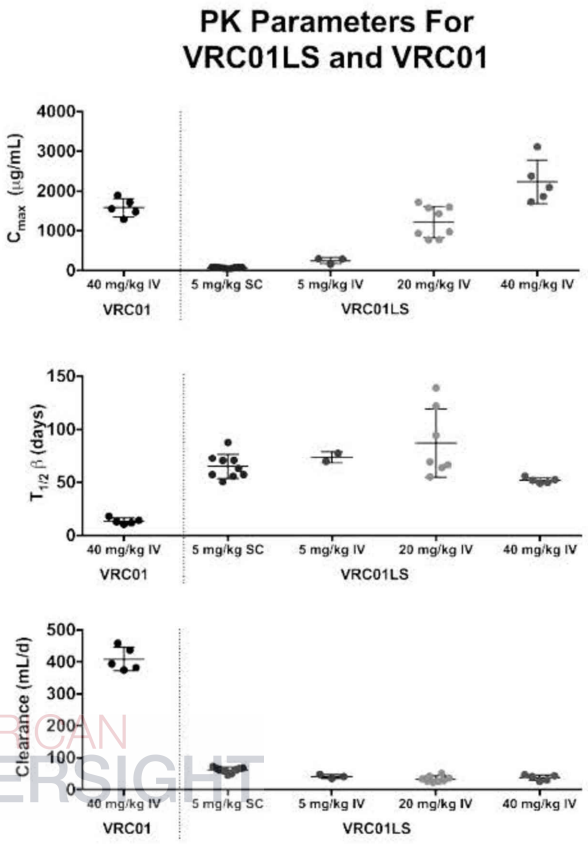
VRC01LS Mean Pharmacokinetic Parameter Values

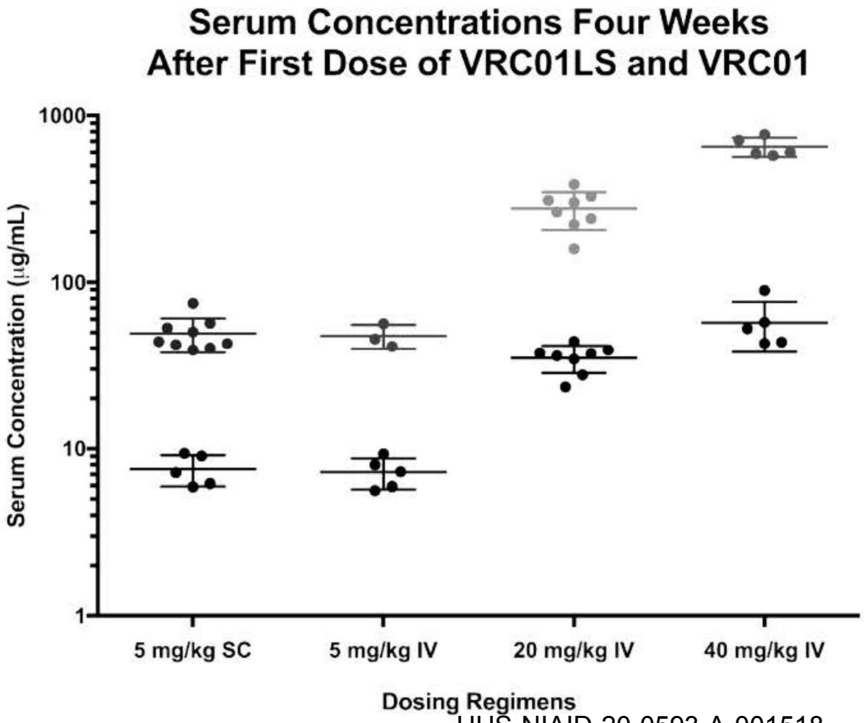
Group and dose	C_{max}	T_{max}	CL	$t_{1/2\beta}$	AUC	4 week post-infusion conc.	12 week post-infusion conc.
				Mean (s.d)			
Intravenous Dosing							
5 mg/kg (n=3)	246 (78)	0.07 (0.08)	40 (7)	83 (11)	4896 (499)*	48 (8)	40 (2.7)*
20 mg/kg (n=8)	1221 (397)	0.2 (0.3)	33 (8)	76 (19)	23368 (5279)*	276 (71)	180 (43)*
40 mg/kg (n=5)	2234 (548)	0.05 (0.02)	38 (9)	55(7)	57099 (13679)	651 (86)	326 (35)
Overall IV (n=16)			36 (8)	71 (18)			
Subcutaneous Dosing							
5 mg/kg (n=9)	69 (15)	9.0 (7.9)	61 (5)+	66 (24)	3777 (814)	49 (11)	25 (5.3)

Includes PK parameters from volunteers who received one or more doses of VRC01LS.* AUC and week 12 values are missing one volunteer from the overall group due to missing week 12 PK samples.+ Value following SC administration represents CL/F.AUC, area under the curve, 0±12 weeks (µg × day/mL); CL, clearance (mL/day); CL/F, apparent clearance; Cmax, maximal serum concentration (µg/mL); Tmax, time to maximal concentration (days); t1/2β, elimination half-life(days)

OVERSIGHT

VRC01 and VRC01LS: PK Parameters and Post Infusion Serum Antibody Concentration





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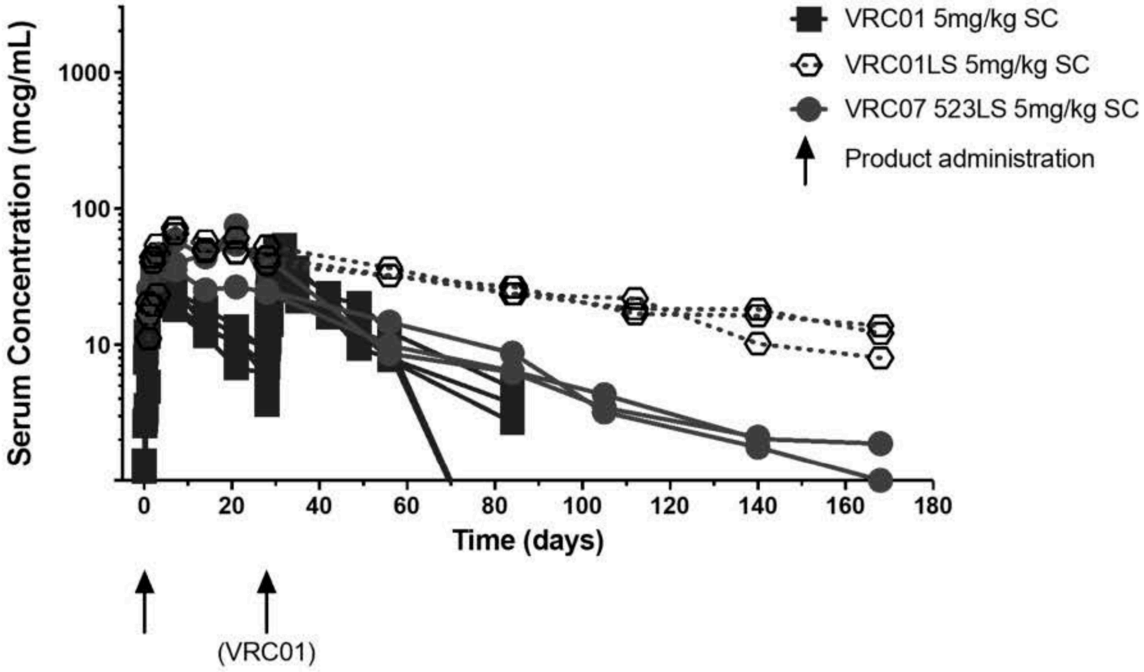
Antibody serum concentration (mcg/ml) of VRC01, VRC01LS, and VRC07-523LS at 5 mg/kg SC after a single administration



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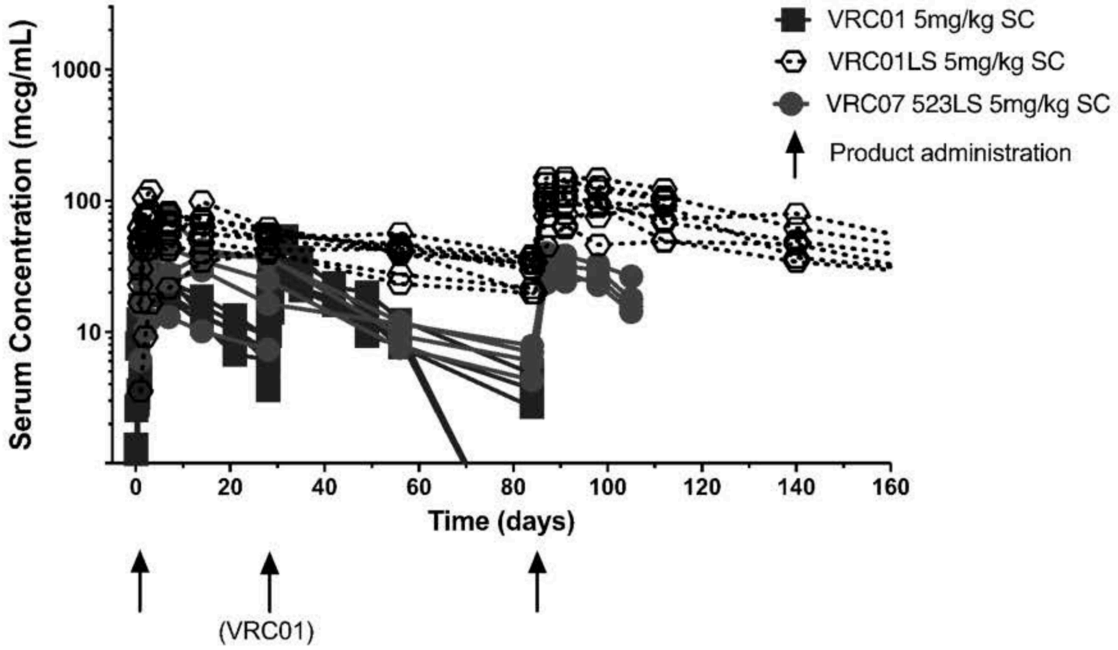
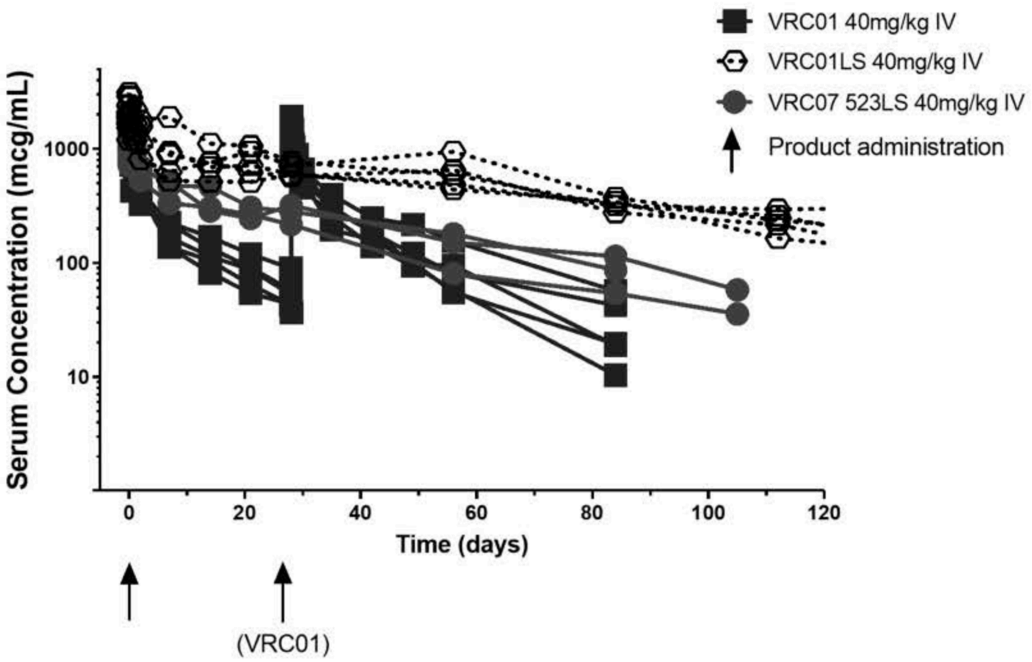
Antibody serum concentration (mcg/ml) of VRC01, VRC01LS, and VRC07-523LS at 5 mg/kg SC after multiple admins



AMERICAN OVERSIGHT
CONFIDENTIAL Interim and Preliminary

Antibody serum concentration (mcg/ml) of VRC01, VRC01LS, and VRC07-523LS at 40 mg/kg IV



AMERICAN OVERSIGHT
CONFIDENTIAL Interim and Preliminary

AMERICAN
OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Thu, 26 Apr 2018 19:45:33 +0000
To: Conrad, Patricia (NIH/NIAID) [E]
Subject: RE: May 3 ASH Brett Giroir visit

Correct

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Thursday, April 26, 2018 2:38 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: FW: May 3 ASH Brett Giroir visit

See highlight -and please advise.

Patricia L. Conrad
Special Assistant to the Director

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From: Chan, Alan (NIH/VRC) [E]
Sent: Thursday, April 26, 2018 2:23 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Daucher, Marybeth (NIH/NIAID) [E] (b)(6) Austin, Sarah (NIH/NIAID) [E] (b)(6)
Subject: Re: May 3 ASH Brett Giroir visit

Correct.

To also confirm, Dr. Fauci will only do his usual NIAID overview slides? In other words Michelle Crank and Jeffrey Boyington will be tasked to do the flu demo in the upstairs lab? Just want to clarify presenters. Occasionally, particularly for past B-roll lab filming, Dr. Fauci has taken the lead in presenting. However, during official tours he typically defers to Drs. Graham (absent this time) / Crank / Boyington to present flu…and we're assuming that's again the case here, yes?

From: "Conrad, Patricia (NIH/NIAID) [E]" (b)(6)
Date: Thursday, April 26, 2018 at 2:03 PM
To: "Chan, Alan (NIH/VRC) [E]" (b)(6)
Cc: "Daucher, Marybeth (NIH/NIAID) [E]" (b)(6) "Austin, Sarah

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(NIH/NIAID) [E]" (b)(6)
Subject: RE: May 3 ASH Brett Giroir visit

Just to clarify a bit more - they will show the molecules on the screen as in past tours right? he was asking...

Patricia L. Conrad
Special Assistant to the Director

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Thursday, April 26, 2018 1:50 PM
To: Chan, Alan (NIH/VRC) [E] (b)(6) Blackburn, Amy (NIH/OD) [E] (b)(6)
Cc: Daucher, Marybeth (NIH/NIAID) [E] (b)(6) Austin, Sarah (NIH/NIAID) [E] (b)(6) McManus, Ayanna (NIH/OD) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6)
Subject: RE: May 3 ASH Brett Giroir visit

Hi Alan –

I know we are still waiting to hear from Amy about the timing but in regard to the tour – I showed the options to ASF and he prefers to keep the tour to universal flu vax. No ebola.

Thanks.

Patricia L. Conrad
Special Assistant to the Director

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From: Chan, Alan (NIH/VRC) [E]
Sent: Wednesday, April 25, 2018 11:42 AM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6) Blackburn, Amy (NIH/OD) [E] (b)(6)
Cc: Daucher, Marybeth (NIH/NIAID) [E] (b)(6) Austin, Sarah (NIH/NIAID) [E] (b)(6) McManus, Ayanna (NIH/OD) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6)
Subject: RE: May 3 ASH Brett Giroir visit

Hi Patti and Amy,

Patti: Thanks.

Amy: So we're on the same planning page, does this look viable?

Agenda (tentative)

10:00	Welcome
10:00 – 10:15	Dr. Fauci NIAID overview slides (w/ time for questions from Dr. Giroir)
10:15 – 10:25	Dr. Koup VRC overview slides (w/ time for questions from Dr. Giroir)
10:25 – 10:30	Head upstairs to lab
10:30 – 10:45	Flu demo: universal flu vaccine development by senior scientists Dr. Michelle Crank and Dr. Jeffrey Boyington
10:45	Group photo (optional) & conclusion

Dr. Mascola was thinking flu would be a high priority topic to present with all the discussion concerning flu vaccines and funding this past season. Another alternate option is we could check the availability of Dr. Nancy Sullivan who has presented an Ebola demo previously. Dr. Giroir was involved in Texas' Ebola response a few years ago. However, for a 45 minute tour it'd be very difficult to do two demos without feeling rushed and barely any time for questions. Your thoughts? Particularly on topic(s)?

Thanks,
Alan

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Wednesday, April 25, 2018 11:20 AM
To: Austin, Sarah (NIH/NIAID) [E] (b)(6) Chan, Alan (NIH/VRC) [E] (b)(6)
Cc: Daucher, Marybeth (NIH/NIAID) [E] (b)(6) Blackburn, Amy (NIH/OD) [E] (b)(6) McManus, Ayanna (NIH/OD) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6)
Subject: RE: May 3 ASH Brett Giroir visit

Hi All –

Putting everyone on the same email so we are all on the same page.

AMERICAN OVERSIGHT

We are holding May 3 10 am – 12 pm for the ASH Giroir visit. I just spoke with Amy Blackburn who is coordinating this for (b)(6) They would like to do "everything" at the VRC. Dr. Collins will have some other meetings that will not include NIAID but then there will be a part where NIAID does their usual meeting with slides and then tour the VRC.

I leave it to Amy to fill you all in but basically as of now – NIAID's portion will be 10 am – 10:45 am. The plan is for ASF to do his overview with slides – Rick Koup will then do his thing and give the tour.

At approx. 10:45 am – Dr. Collins and others will have their meetings but still at the VRC.

Will leave it to Amy to take over…..

Thanks to all.

Patricia L. Conrad
Special Assistant to the Director

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From: Austin, Sarah (NIH/NIAID) [E]
Sent: Wednesday, April 25, 2018 9:01 AM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6) Chan, Alan (NIH/VRC) [E] (b)(6)
Cc: Daucher, Marybeth (NIH/NIAID) [E] (b)(6)
Subject: Re: May 3

Good morning all –

AMERICAN OVERSIGHT

Rick Koup is available on May 3, but John and Barney are out of the office.

Thank you,
Sarah

From: "Chan, Alan (NIH/VRC) [E]" (b)(6)
Date: Tuesday, April 24, 2018 at 6:01 PM
To: "Austin, Sarah (NIH/NIAID) [E]" (b)(6) "Conrad, Patricia (NIH/NIAID) [E]" (b)(6)
Cc: "Daucher, Marybeth (NIH/NIAID) [E]" (b)(6)
Subject: Re: May 3

Hi Patti,

I just heard it as breaking news from Tara Schwetz at a Fishers Lane policy meeting I was at this afternoon. She'd just heard of it that hour herself and said NIAID would like to plan for Dr. Giroir to tour VRC.

We'll await further details while gauging our staff's availability and plans.

Thanks,
Alan

From: "Austin, Sarah (NIH/NIAID) [E]" (b)(6)
Date: Tuesday, April 24, 2018 at 4:20 PM
To: "Conrad, Patricia (NIH/NIAID) [E]" (b)(6)
Cc: "Daucher, Marybeth (NIH/NIAID) [E]" (b)(6) "Chan, Alan (NIH/VRC) [E]" (b)(6)
Subject: Re: May 3

Thanks for the heads up, Patti. This is the first I have heard of anything. I'll note it on John's calendar and alert Rick and Barney, too.

Sarah

From: "Conrad, Patricia (NIH/NIAID) [E]" (b)(6)
Date: Tuesday, April 24, 2018 at 2:35 PM
To: "Austin, Sarah (NIH/NIAID) [E]" (b)(6) "Daucher, Marybeth (NIH/NIAID) [E]" (b)(6) "Chan, Alan (NIH/VRC) [E]" (b)(6)
Subject: May 3

(b)(6) just called us about a meeting/vrc tour for the HHS Asst Sec of Health Brett Giroir....have you all been contacted about this yet? If not

AMERICAN OVERSIGHT

– can you please put it on your radar. I will discuss with ASF today. I am guessing it will be the usual slide show then tour but will see…..

https://en.wikipedia.org/wiki/Brett_Giroir

thx

Patricia L. Conrad
Public Health Analyst and
Special Assistant to the Director
National Institute of Allergy and Infectious Diseases
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From: Glass, Roger (NIH/FIC) [E]
Sent: Thu, 26 Apr 2018 14:30:23 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: (b)(6)
Subject: FW: my talk
Attachments: Fogarty 50th anniversary April 25, 2018.pptx

Tony

Great presentation this morning. Nice to have a little science in these meetings!

Attached is Bill's presentation for the Fogarty Symposium next Tuesday.. Bill will be at the dinner at La Ferme Monday night at 6 pm. Hope you can join as well.

I think Bill will ask you about your visit to Haiti to see the amazing things GHESKIO is doing with XDR-TB, zika, cholera vaccines, chik, and of course HIV!

Roger

From: Jean W Pape
Sent: Wednesday, April 25, 2018 12:19 PM
To: Glass, Roger (NIH/FIC) [E]
Subject: RE: my talk

Roger,

Please share with Tony. I have added what I was planning to say about GHESKIO and the Fogarty. I believe that the time is now for implementation science to help scale-up widely the proven scientific findings I order to make the end of AIDS happen.

Best,

Bill

JEAN WILLIAM PAPE, MD
Howard and Carol Holtzmann Professor of Clinical Medicine,
Center for Global Health, Division of Infectious Diseases, Department of Medicine,
Weill Cornell Medical College, New York, New York, USA
402 East 67th Street, New York, NY 10065
Tel (Office): 646-962-8140/8146

Director,
Les Centres GHESKIO,
33, Blvd. Harry Truman, Port-au-Prince, Haïti.
Tel (Office): 011-509-2940-1431; 011-509- 2942-6327

AMERICAN OVERSIGHT

Voip:(b)(6) ;(b)(6)

From: Glass, Roger (NIH/FIC) [E] (b)(6)
Sent: Tuesday, April 24, 2018 11:43 PM
To: Jean W Pape
Subject: Re: my talk

Bill

This looks like a Big Idea! An aspiration that you want to put into practice....like polio eradication 5 decades ago! tony is the moderator for your session, so with your permission, can I share this with him.

Do include a few personal words about Fogarty and gheskio since you are our poster child for having 4-5 generations of researchers in a line of training-.

Looking forward to seeing you Monday night for dinner at la(b)(6) This will be a fun and thoughtful event.

Roger

Sent from my iPad

On Apr 24, 2018, at 6:01 PM, Jean W Pape (b)(6) wrote:

Roger,

You must be quite busy as the meeting date is getting close.
I wanted to give you a preview of my talk. First of all I wanted to focus on Latin America and the Caribbean as the first thing to do is to know your epidemic: the populations most at risk among the new infections.
I also wanted to show that we have the means to prevent transmission and decrease mortality to an accepted level. The science is there but what is needed is the science of implementation.

Let me know what you think.

Best,

Bill

JEAN WILLIAM PAPE, MD
Howard and Carol Holtzmann Professor of Clinical Medicine,
Center for Global Health, Division of Infectious Diseases, Department of Medicine,
Weill Cornell Medical College, New York, New York, USA
402 East 67th Street, New York, NY 10065
Tel (Office): 646-962-8140/8146

Director,

AMERICAN OVERSIGHT

Les Centres GHESKIO,
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Tel (Office): 011-509-2940-1431; 011-509- 2942-6327
Voip: (b)(6)

AMERICAN OVERSIGHT

AIDS accomplishments and Research needs to end AIDS in Latin America/Caribbean

Jean William Pape MDHoward and Carol Holtzmann Professor of Clinical Medicine, Center for Global Health, Division of Infectious Diseases, Department of Medicine, Weill Cornell Medical College, New York, New York, USA Director, Les Centres GHESKIO, Port-au-Prince, Haïti.

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Fogarty 50th Anniversary Symposium, NIH Campus, Bethesda, Maryland May 1, 2018

HHS-NIAID-20-0593-A-001532

Les Centres GHESKIO (May 2, 1982)

- HIVAIDS/STI, TB Comprehensive Center for:Translational research: 2 publications/month Training: largest post graduate training center in Haiti Services: one of the largest AIDS and TB centers in the Americas Extended to diarrheal diseases, malaria, dengue, HPV, nutrition…Post-earthquake: global health aimed at the poor: care for IDP, primary, vocational, education and microcredit programsPartnership: Ministry of Health and 116 local institutionsGranted status of "Public Utility " by Haitian government (2000)Strong international collaboration: Cornell, F. Mérieux… Continuous support from NIH (1983), Fogarty (1988): 4th generations of Fogarty trainees:>100 trainees

AMERICAN OVERSIGHT

HHS-NIAID-20-0593-A-001533

HIV/AIDS IN LATIN AMERICA THE CARIBBEAN (LAC) REGIONAL OVERVIEW

Latin America and Caribbean (2016)

2.1 million people living with HIV

0.5% adult HIV prevalence

115,000 new HIV infections

45,400 AIDS-related deaths

56% adults on antiretroviral treatment

53% children on antiretroviral treatment

Source: UNAIDS Data 2017

- **Elimination of transmission: Complete cessation of incidence in a geographically defined area. Because the disease-causing agent persists, elimination requires ongoing interventions to maintain. Can we prevent vertical transmission (PMTCT), transmission by blood/blood products and sexual transmission? Elimination as a public health problem: Reduction of incidence and morbidity below a specific (globally defined) level. Can we reduce incidence, mortality/morbidity to an accepted level?**

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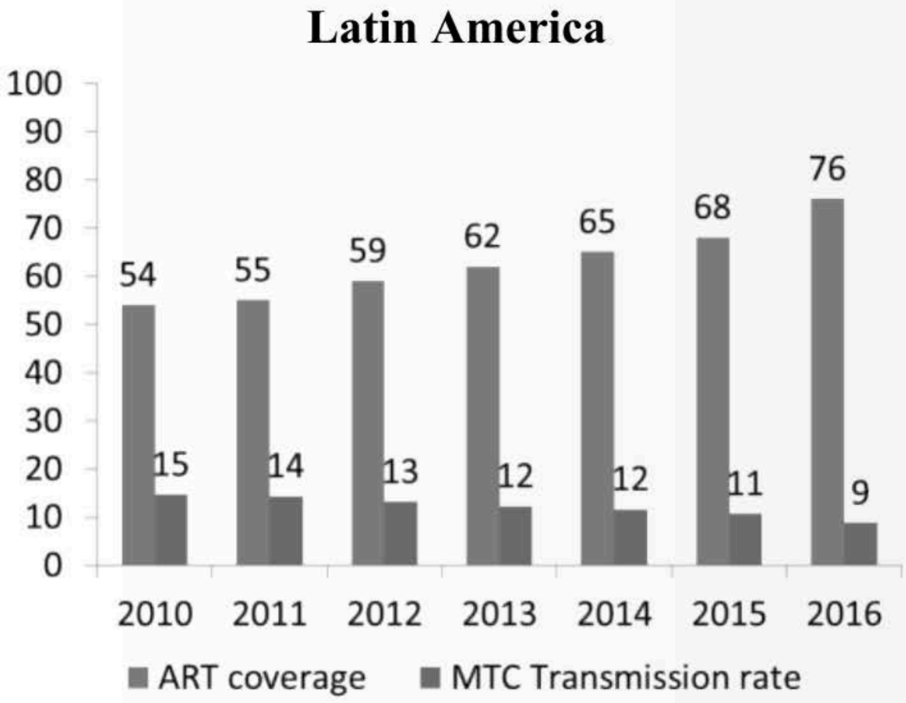
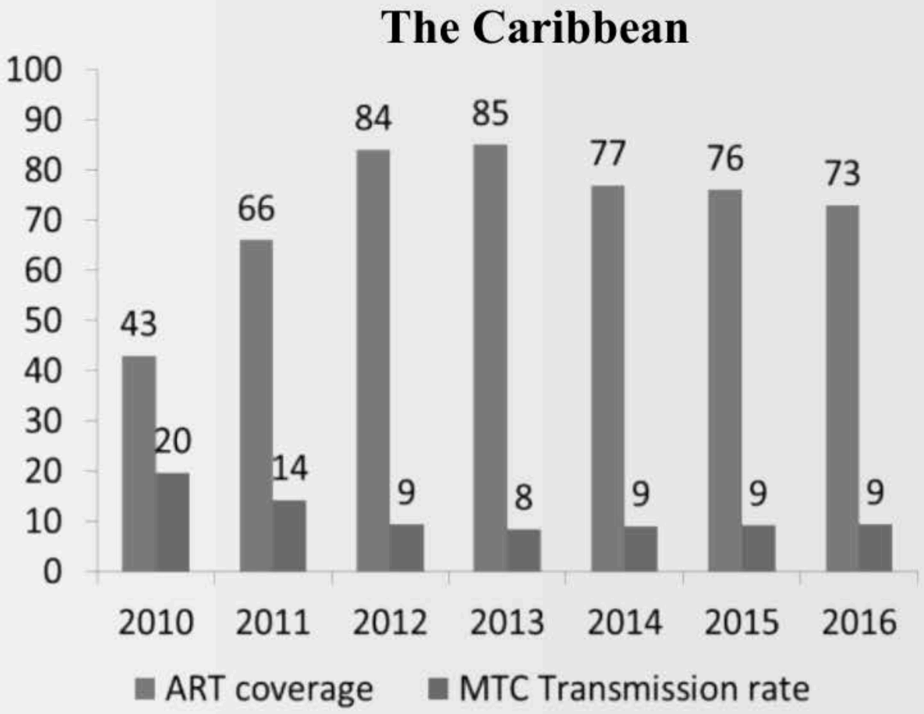
Pregnant Women on Antiretroviral Treatment to Prevent Mother to Child Transmission (%), 2010-2016





WHO Validation of the Elimination of MTCT of HIV and syphilis in LAC

Cuba

Cayman Islands Anguilla

Montserrat St Kitts and Nevis

Antigua and Barbuda Bermuda

Cayman Islands

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Pan American Health Organization

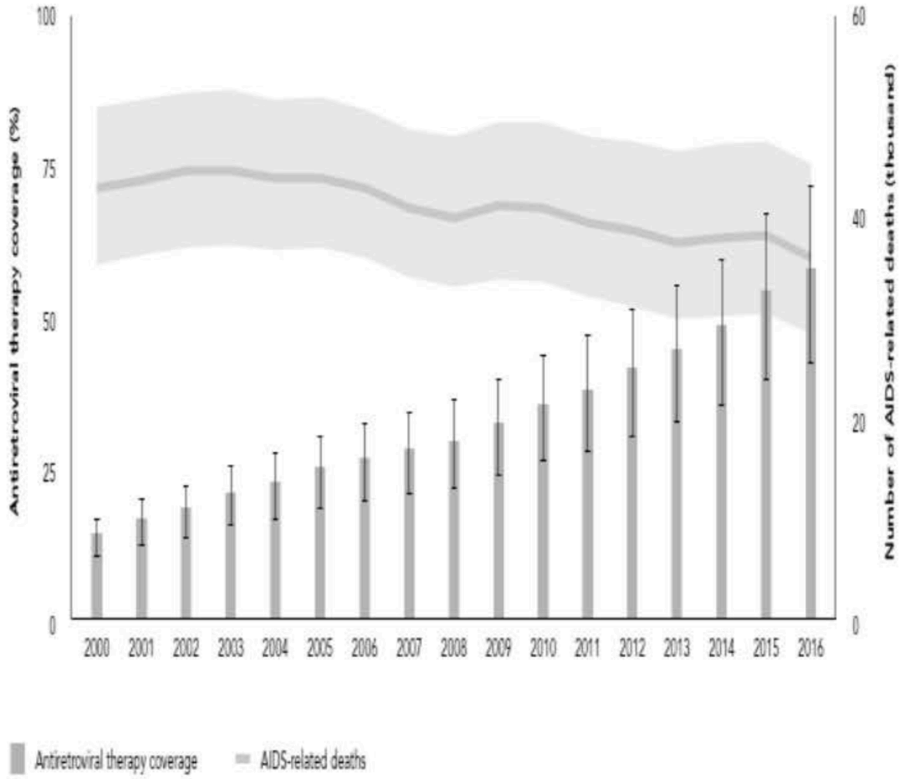
World Health Organization

Source: UNAIDS Spectrum estimates 2017. Note: Estimates are rounded

ART Coverage and AIDS-related deaths in LAC

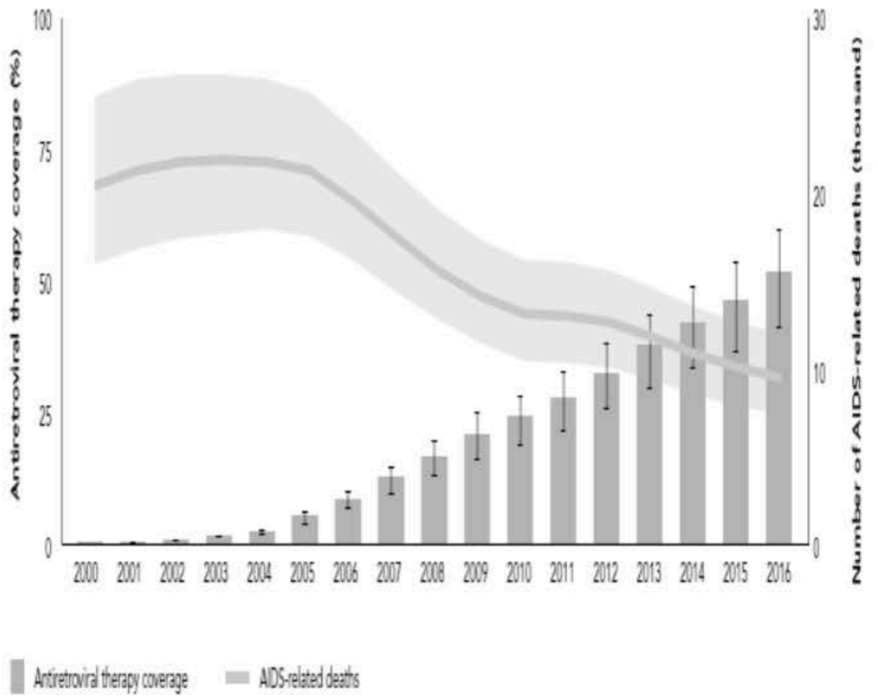
Latin America

12% reduction of AIDS-related mortality



The Caribbean

45% reduction of AIDS-related mortality



2016: 1/3 of new HIV diagnoses are in patients with a baseline CD4 of <200 mm3/mL

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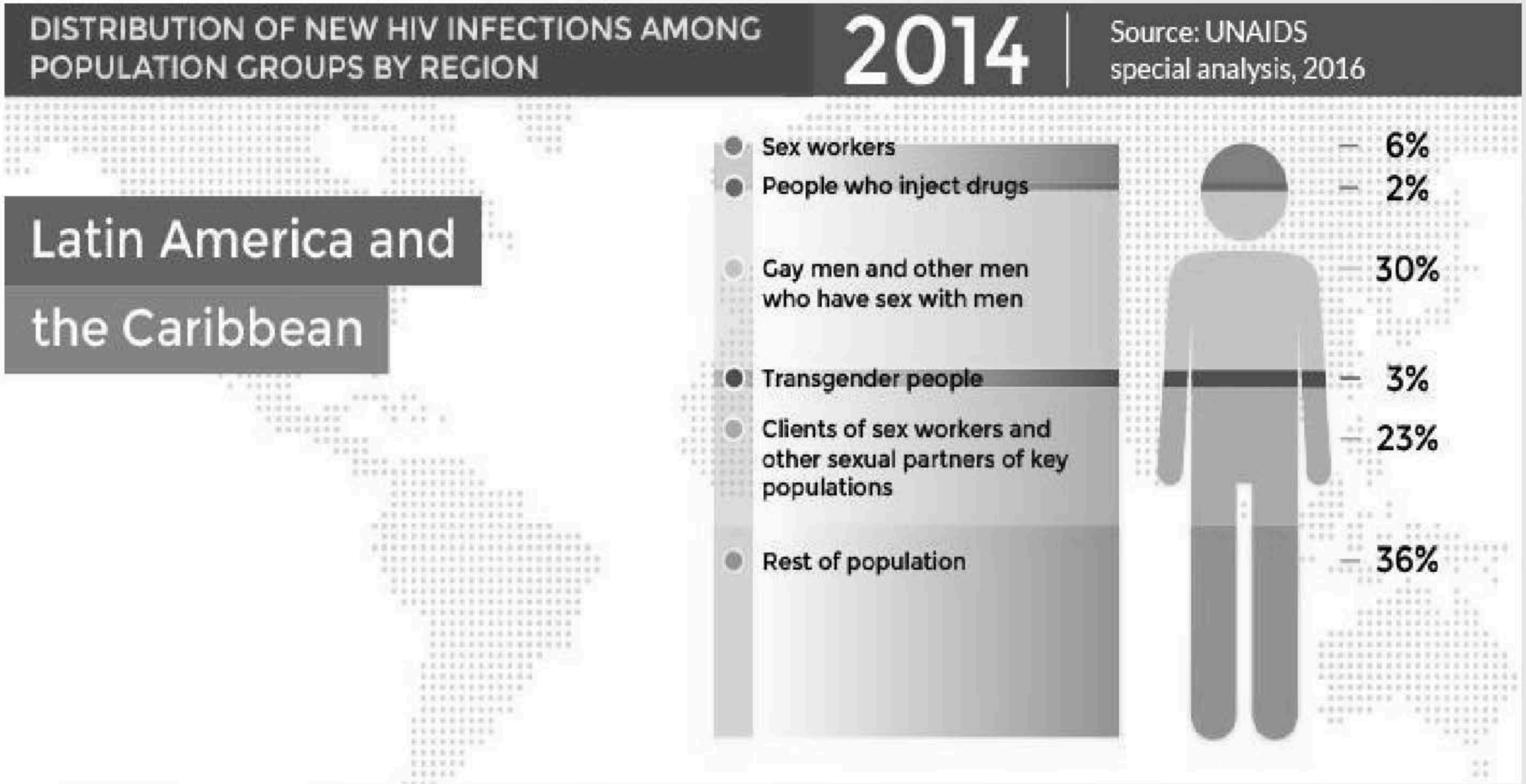
Characteristics of new HIV infections in LAC



64% of new HIV infections are in key populations and their clients

15-24 y/o:1/3 new infections in the Caribbean and LA

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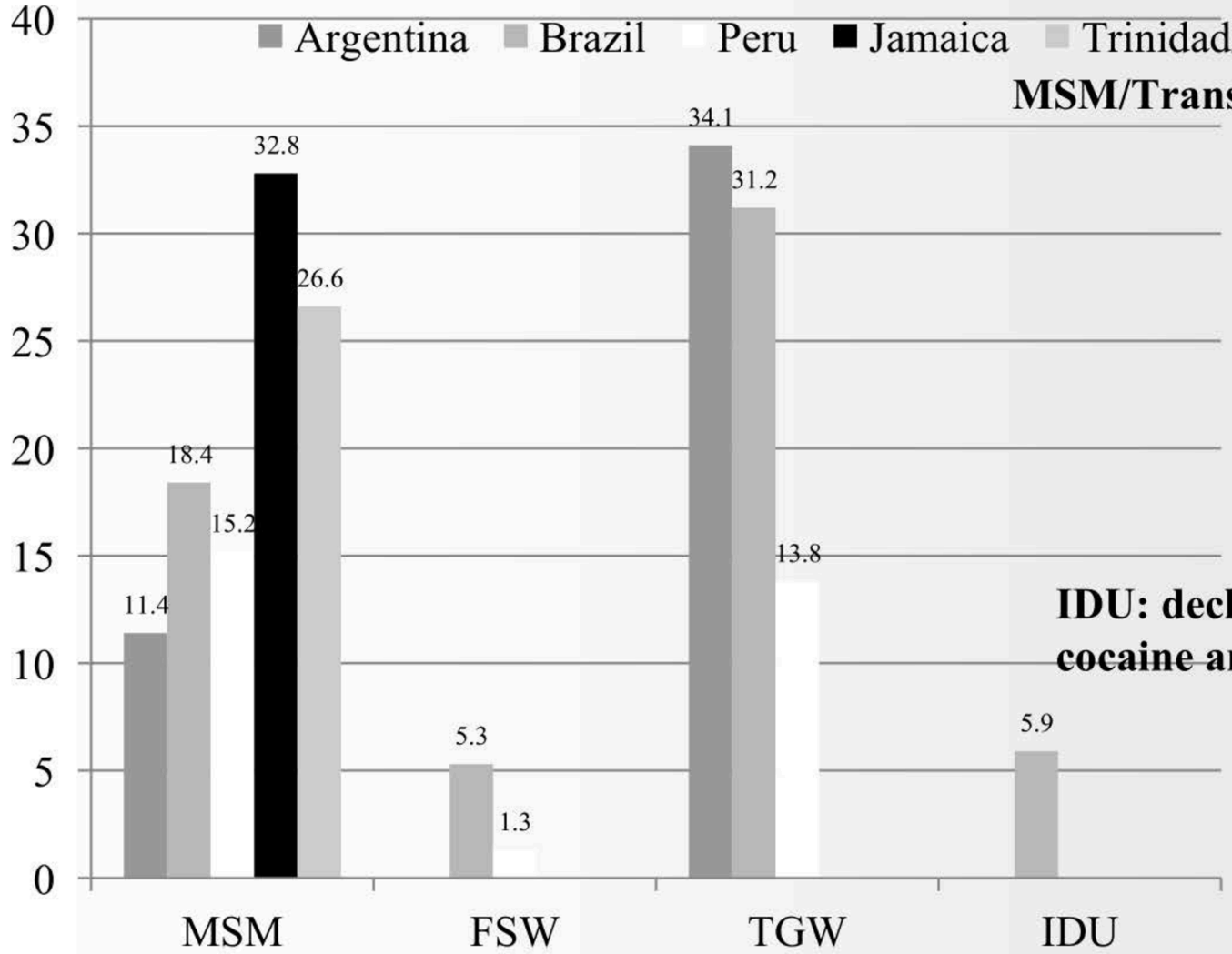
From www.avert.org

HIV Prevalence in Key Populations in LAC



MSM/Transgender: **Low rate**

IDU: declined for non injecting cocaine and derivatives

1. UNAIDS Estimates 2017; 2. Kerr et al, 2017; 3. Szwarcwald et al, 2017; 4. Dos Ramos-Farias et al, 2011; [illegible] Bastos et al, 2013

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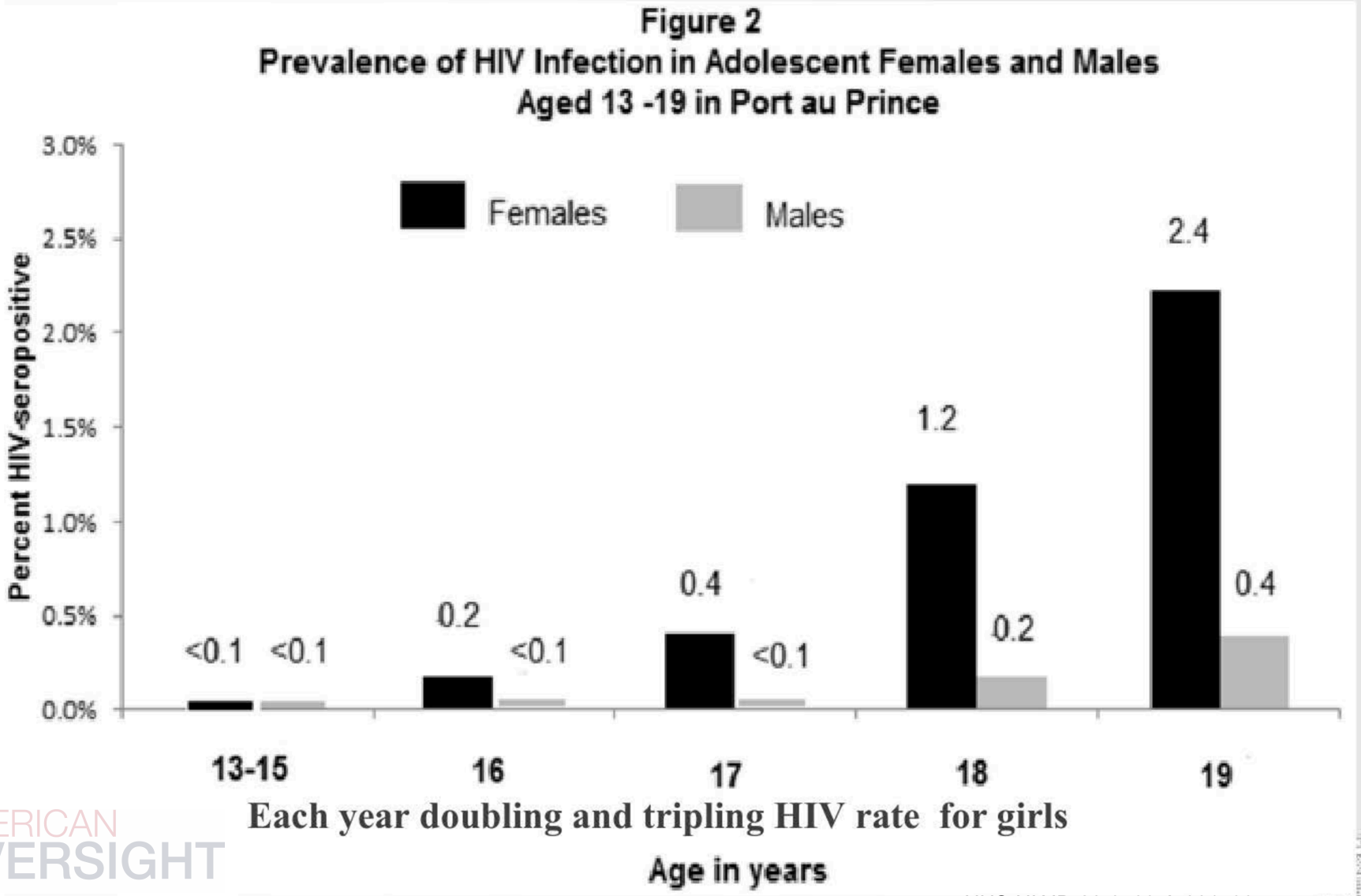
HIV Prevalence in Adolescents in Haiti



AMERICAN OVERSIGHT

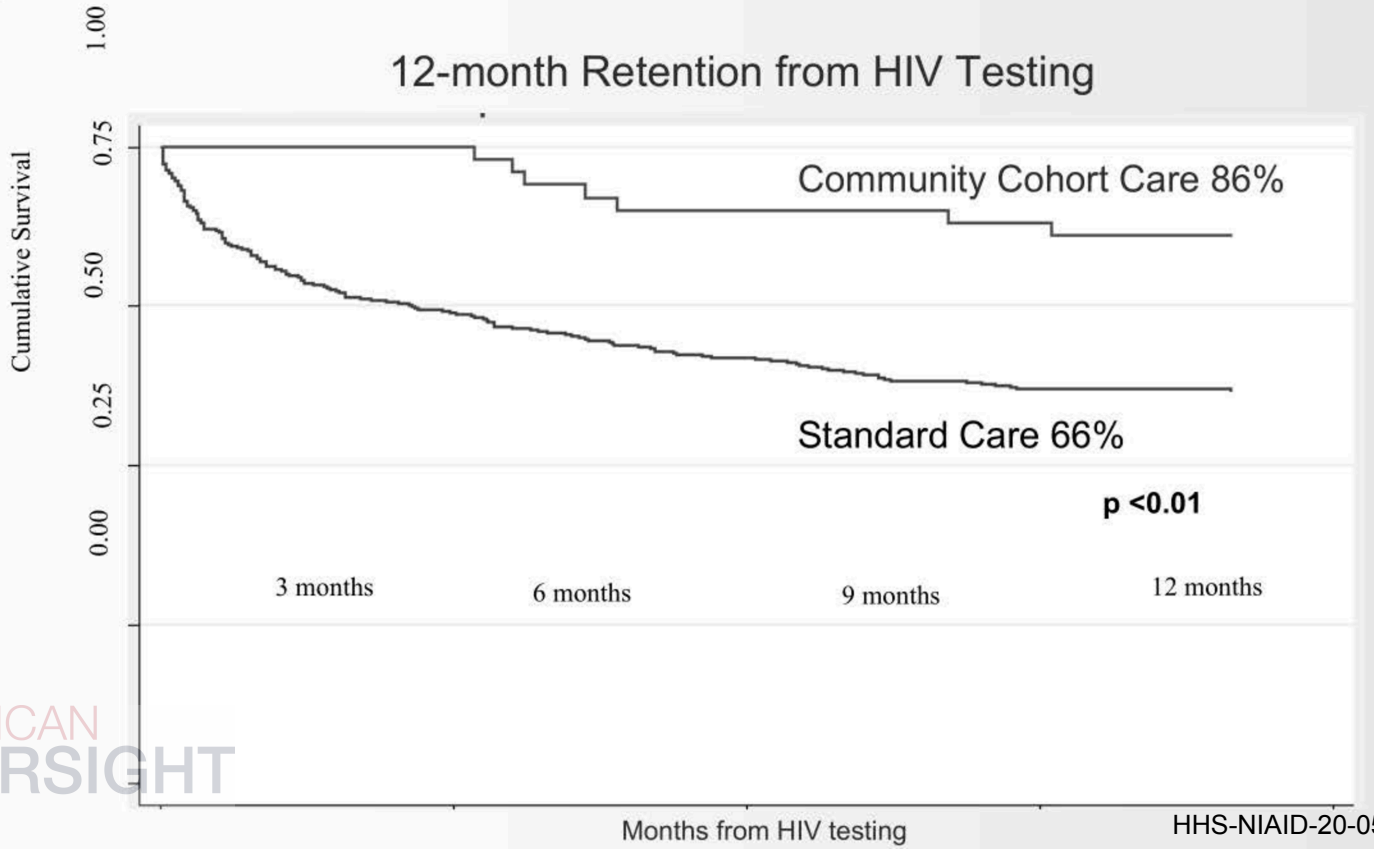
AIDS PATIENT CARE and STDs
Volume 30, Number 8, 2016
© Mary Ann Liebert, Inc.
DOI: 10.1089/apc.2016.0102

Community-Based HIV and Health Testing for High-Risk Adolescents and Youth

Lindsey K. Reif, MS, MPH,[1,*] Vanessa Rivera, BS,[1,*] Bianca Louis, MSW,[2] Rachel Bertrand, MD,[2] Mireille Peck, MD,[2] Benedict Anglade, BS,[2] Grace Seo, BS,[1] Elaine J. Abrams, MD,[3,4] Joan W. Pape, MD,[1,2] Daniel W. Fitzgerald, MD,[1] and Margaret L. McNairy, MD, MSc[1,5]

98% acceptability; recruit high risk ado: 2.6% HIV rate vs. 0.8% with previous method

- Monthly cohort session 6-8 vs monthly individual clinic visit: help social isolationAll services provided in group setting in the community instead of the clinicAll services in cohort group session with 1 nurse instead of sequential health care providers



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Percentage of LAC countries with public policies for provision of the HIV prevention services by key population and selected services

Service or intervention	MSM (%)	FSW (%)	Transgender women (%)
HIV testing and counseling	100	97	94
STI diagnosis and treatment	90	91	84
PrEP	6	6	6
PEP	39	39	39
Condoms	100	96	81
Lubricants	89	89	83
Antiretroviral treatment (ART) for all	45	45	45
Peer-led community outreach activities	89	86	83
Sexual health information and education	100	96	91

Source: *PAHO. Country responses to the HIV Prevention Survey, May 2017.;* **Note:** *Percentages based on the response of 31 or 33 countries as of July 2017.*

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Summary: What is needed to end AIDS in LAC?

- We have the tools to end AIDS as a public threat by 2030 if :Have political/financial commitment for both care and preventionImplementation science is focused on:Key populations: MSM/TRANS, sex workers/clients with Community-centered approach to increase HIV diagnoses and implement a wide range of services: test/treat Prep, PEP, circumcision, STI control, condoms..) Adolescents/young adults through comprehensive services Prevent late comers with advanced AIDS using community approach and test/treatAddress poverty, food insecurity, drivers of epidemic in many placesInvest in human resources, technical capacity for low income countries: the support of the Fogarty has been invaluable in Haiti

AMERICAN OVERSIGHT

From: Goosby, Eric
Sent: Thu, 26 Apr 2018 05:41:50 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Reid, Michael;Eisinger, Robert (NIH/NIAID) [E];Doepel, Laurie (NIH/NIAID) [E]
Subject: Re: Lancet Commission final pre-submission draft Review
Attachments: image001.gif, image002.png

Tony, Laurie and Bob;
Can't thank you all enough. Laurie' s input unplugged our perseverations so we could deliver clear messages. Bob was terrific in defining the details, as usual. Tony, your support with this once again makes me realize how lucky I am to have your friendship.
Warm regards and deep gratitude,
Eric

Sent from my iPhone

On Apr 25, 2018, at 10:33 PM, Fauci, Anthony (NIH/NIAID) [E] (b)(6) wrote:

Mike:
Thanks for the note. You and Eric did a great job on this prodigious task. We were pleased to be able to help in a small way.
Best regards,
Tony

From: Reid, Michael (b)(6)
Sent: Wednesday, April 25, 2018 5:28 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6) Goosby, Eric (b)(6)
Cc: Eisinger, Robert (NIH/NIAID) [E] (b)(6) Doepel, Laurie (NIH/NIAID) [E] (b)(6)
Subject: Re: Lancet Commission final pre-submission draft Review

Dr. Fauci,

Firstly, many thanks for your willingness to review this draft. I appreciate that you are exceptionally busy and am grateful that you took the time to review the work.

Secondly, I want to extend thanks for allowing us to avail of Bob and Laurie's knowledge and skills towards this effort.

I intend to spend the next few hours going through all of your comments and edits systematically, and will incorporate where appropriate. In addition, I will revise the figure 2.0.1 based on your input.

I am confident that we can considerably improve the draft based on your inputs, as well as those of the other commissioners who volunteered feedback.

Kind regards,

AMERICAN OVERSIGHT

Mike

From: "Fauci, Anthony (NIH/NIAID) [E]" (b)(6)
Date: Wednesday, April 25, 2018 at 10:55
To: "Reid, Michael" (b)(6) Eric Goosby (b)(6)
Cc: "Eisinger, Robert (NIH/NIAID) [E]" (b)(6) "Doepel, Laurie (NIH/NIAID) [E]" (b)(6)
Subject: Lancet Commission final pre-submission draft Review

Eric and Mike:

Thank you for the opportunity to review the entire draft Report. Your efforts to compile the feedback from all of the Commissioners into this draft should be applauded.

The first attachment labeled "Lancet TB Report Comments 4.23.18" lists significant comments and suggested edits in each section. I wanted to bring your attention a few of the most important of these, highlighted below:

- Executive Summary - Vision Statement – I would propose the following: "With bold leadership, accelerated research and development, implementation of evidence-based strategies, and a shared responsibility, we can end TB within a generation."
- Executive Summary - Priority 1 should be revised to include as the next to the last sentence "Special attention should be focused on TB and HIV coinfection."
- Executive Summary - Table 1 should be used rather than Figure 1.
- Section 2 – We propose an additional priority for this Section should be added -- "Invest in critical research on TB and HIV coinfection."
- Section 2 – Figure 2.0.1 should be used and not Figure 2.2.1. Two modifications should be considered to Figure 2.0.1 including: 1) the "Industry and Academic Partnerships" bar on the schematic could be larger reflecting its importance in the product development process; and 2) this bar on the schematic could be narrower in the "Basic Science/Biomarker" region and gradually get wider. The attached schematic which was provided previously illustrates the latter modification.

Additional comments and suggested edits can be found in each section of the Report in the tracked changes versions at the links below. As noted, in a few instances, figures/tables cited in the narrative are missing, or figures/tables that appear in the report are not cited in the narrative.

Executive Summary link https://file.ac/4vvxNEHLGyY/
Section 0 link https://file.ac/e4dGSd7FYVE/
Section 1 link https://file.ac/lpG4ovDsWYw/
Section 2 link https://file.ac/xQCyttQzkjE/

AMERICAN OVERSIGHT

Section 3 link https://file.ac/D4R94Fn5hjk/
Section 4 link https://file.ac/Ue7pZHn3PoY/
Panels link https://file.ac/eJRGAfikEic/

I suggest that the non-Commissioners who assisted in developing the Report should be clearly listed as "Contributors" immediately below the list of Commissioners who are the formal "authors". However, whatever you decide is fine with me.

If we can provide any additional information or assistance, please let us know.

Best,

Tony

Anthony S. Fauci, MD
Director
National Institute of Allergy and Infectious Diseases
Building 31, Room 7A-03
31 Center Drive, MSC 2520
National Institutes of Health
Bethesda, MD 20892-2520
Phone: (b)(6)
FAX: (301) 496-4409
E-mail: (b)(6)
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---

rom: Reid, Michael (b)(6)
Sent: Friday, April 20, 2018 3:14 AM
To: Goosby, Eric (b)(6)
Cc: Dean Jamison (b)(6) Soumya Swaminathan (b)(6)
Subject: Re: Lancet Commission final pre-submission draft Review

Dear Commissioners,

AMERICAN OVERSIGHT

Thank you to all those of you that have contributed to revising the report and responding to the external reviewer comments. In particular, thanks to the teams from NIH and FIND for their help pulling pieces together!

Please find attached a zip file containing the current draft [minus a few pending analyses]. The full report – in 6 sections – can also be downloaded:

From Box: https://ucsf.box.com/s/uqzzpsza4w86x2rmulvyua0bufylpsgi

From Dropbox: https://www.dropbox.com/sh/9se9699agk5s5ck/AAAXQKMeELK728MoCzm-Oifua?dl=0

In order to ensure that we have the best chance of getting the report published in time for the HLM, The Lancet have requested that we formally submit by **April 27th**. I fully appreciate that this means everyone has a short time to review and respond. Nonetheless, we would be grateful for your comments/edits by **April 24** if possible.

In particular, please review the executive summary, in particular the 'vision statement' and the more concise recommendations (5 rather than 10), which align with the HLM 'key asks'.

Eric and I welcome your feedback via email. If possible, share any substantive recommendations/comments in the email, rather than track changes to the documents, to ensure we don't miss any major comments. However, feel free to use track changes if that is your preference. I can also be available most of next week (April 23-26) by 'phone or Skype to discuss questions/clarifications/concerns.

Finally, several of you partnered with non-commissioners to complete writing/analytic products – If you feel these colleagues should be considered as co-authors on the publication, *and you haven't already reached to me to discuss*, please let me know.

Thank you all for your continued support and collaboration in this effort and the important roles you each play in 'building a TB-free world.'

Best regards,

Mike

Lancet Commission on TB, Secretariat

From: "Reid, Michael" (b)(6)
Date: Sunday, April 15, 2018 at 06:22
To: Eric Goosby (b)(6)
Subject: Re: Lancet Commission Conf calls - week of April 16-20

Dear Commissioners,

AMERICAN OVERSIGHT

Apologies for the delay in failing to circle back on this email from David sooner. Unfortunately, we were unable to find any date/time that worked for more than 2 or 3 people. With this in mind, Eric and I have decided to forgo a conference call for all Commissioners.

We are aiming to formally submit a revised draft of the Commission, as well as responses to all the external reviewers' comments, to The Lancet by **April 27**. In advance of that date, I intend to share a pre-submission draft of the full report to all Commissioners this coming Wed/Thurs [**April 18/19**]. I kindly ask that you all take time to review and make edits/comments – sending back any comments/edits no later than April 23.

I can personally be available for one-on-one calls on April 23 and 24 for anyone that wants to give feedback over the 'phone. Please email me individually if you would like to connect on either day.

Thanks for all of your input, particularly those of you that have been re-writing or reviewing subsections of the report over the last 2 weeks.

Kind regards,

Mike Reid
(on behalf of the Commission Secretariat)

From: David McKey (b)(6)
Date: Wednesday, April 4, 2018 at 09:52
To: David McKey (b)(6)
Cc: "Reid, Michael" (b)(6)
Subject: Lancet Commission Conf calls - week of April 16-20

<image001.gif>
Dear Commissioners,
As a follow-up to our recent meeting in New Delhi, it was agreed that we would arrange for two conference calls the week of April 16-20 to give an update on the progress and discuss any last minute details before the report is submitted the end of April.

Please find the link for a doodle poll indicating possible times for a call. Please select the two dates and times that work the best for you.
(b)(6)

AMERICAN OVERSIGHT

If you plan on submitting expenses for reimbursement, please send as soon as possible. As an FYI, I will be out on holiday 10-22 April. Expenses not received before 6 April will be submitted upon my return to the office.

And, attached is the picture from our meeting.

Thank you,
David

David McKey
Office of the UN Secretary-General's Special Envoy on Tuberculosis
Global Health Delivery & Diplomacy
Institute for Global Health Sciences | UCSF
550 16th Street | 3rd Floor | Box 1224
San Francisco, California 94158

(b)(6)
(b)(6)

<image002.png>

AMERICAN OVERSIGHT

UCSF Institute for Global Health Sciences

AMERICAN OVERSIGHT

-A-001549

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Thu, 26 Apr 2018 02:34:27 +0000
To: 'Robin Nandy'
Subject: RE: enjoyed our chat -- hopefully more to come

Robin:
Thanks for the note. I look forward to the opportunity to work with you in the future.
Best regards,
Tony

From: Robin Nandy (b)(6)
Sent: Wednesday, April 25, 2018 5:36 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6) Steven Johnson (b)(6)
Cc: Conrad, Patricia (NIH/NIAID) [E] (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Subject: RE: enjoyed our chat -- hopefully more to come

Dear Tony

It was indeed an honor to be on a panel with you – a true medical and public health legend. Look forward to being in touch with you and your team and hope we can collectively move the agenda of GVAP 2.0 forward.

Regards

Robin

Dr. Robin Nandy
Principal Advisor & Chief of Immunizations
UNICEF House
3 United Nations Plaza
New York, NY 10017
USA
Tel: (b)(6)
Cell: (b)(6)
Email: (b)(6)

From: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Sent: Wednesday, April 25, 2018 11:16 AM
To: Steven Johnson (b)(6) Robin Nandy (b)(6)

AMERICAN OVERSIGHT

Cc: Conrad, Patricia (NIH/NIAID) [E] (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Subject: RE: enjoyed our chat -- hopefully more to come

Steven:
Thanks for the note. It was a lot of fun being on the panel with you, Robin and Travis. I would be happy to help out in your upcoming project. I look forward to hearing from you about it.
Best regards,
Tony

Anthony S. Fauci, MD
Director
National Institute of Allergy and Infectious Diseases
Building 31, Room 7A-03
31 Center Drive, MSC 2520
National Institutes of Health
Bethesda, MD 20892-2520
Phone: (b)(6)
FAX: (301) 496-4409
E-mail: (b)(6)
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From: Steven Johnson (b)(6)
Sent: Wednesday, April 25, 2018 9:51 AM
To: (b)(6) Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: enjoyed our chat -- hopefully more to come

Tony, Robin -- I just wanted to say how much I enjoyed our chat at the Metropolitan Club on Monday night. Was a real honor being on stage with both of you!

I'd like to be back in touch with you both later this year -- one of my upcoming projects (besides the cholera musical!) is a book and TV series about the last hundred years of global life expectancy gains. (The plan is to release the book/show in 2020, the centennial of the end of the spanish flu.) The goal is to explain/celebrate just how we have managed to achieve such extraordinary increases in average lifespan, and speculate on the tools/institutions we are going to need to continue those trends over the next century....

For obvious reasons, you would both be invaluable sources of wisdom and experience for this project. So I will be back in touch when we start working on it in earnest...

best,

Steven

AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Wed, 25 Apr 2018 21:33:18 +0000
To: Reid, Michael;Goosby, Eric
Cc: Eisinger, Robert (NIH/NIAID) [E];Doepel, Laurie (NIH/NIAID) [E]
Subject: RE: Lancet Commission final pre-submission draft Review

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Mike

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Date: Wednesday, April 25, 2018 at 10:55
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Cc: "Eisinger, Robert (NIH/NIAID) [E]" (b)(6) "Doepel, Laurie (NIH/NIAID) [E]" (b)(6)
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Eric and Mike:

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- Executive Summary - Table 1 should be used rather than Figure 1.
- Section 2 – We propose an additional priority for this Section should be added -- "Invest in critical research on TB and HIV coinfection."
- Section 2 – Figure 2.0.1 should be used and not Figure 2.2.1. Two modifications should be considered to Figure 2.0.1 including: 1) the "Industry and Academic Partnerships" bar on the schematic could be larger reflecting its importance in the product development process; and 2) this bar on the schematic could be narrower in the "Basic Science/Biomarker" region and gradually get wider. The attached schematic which was provided previously illustrates the latter modification.

Additional comments and suggested edits can be found in each section of the Report in the tracked changes versions at the links below. As noted, in a few instances, figures/tables cited in the narrative are missing, or figures/tables that appear in the report are not cited in the narrative.

Executive Summary link https://file.ac/4vvxNEHLGyY/
Section 0 link https://file.ac/e4dGSd7FYVE/
Section 1 link https://file.ac/lpG4ovDsWYw/
Section 2 link https://file.ac/xQCyttQzkjE/
Section 3 link https://file.ac/D4R94Fn5hjk/
Section 4 link https://file.ac/Ue7pZHn3PoY/
Panels link https://file.ac/eJRGAfikEic/

I suggest that the non-Commissioners who assisted in developing the Report should be clearly listed as "Contributors" immediately below the list of Commissioners who are the formal "authors". However, whatever you decide is fine with me.

If we can provide any additional information or assistance, please let us know.
Best,
Tony

AMERICAN OVERSIGHT

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rom: Reid, Michael (b)(6)
Sent: Friday, April 20, 2018 3:14 AM
To: Goosby, Eric (b)(6)
Cc: Dean Jamison (b)(6) Soumya Swaminathan (b)(6)
Subject: Re: Lancet Commission final pre-submission draft Review

Dear Commissioners,

Thank you to all those of you that have contributed to revising the report and responding to the external reviewer comments. In particular, thanks to the teams from NIH and FIND for their help pulling pieces together!

Please find attached a zip file containing the current draft [minus a few pending analyses]. The full report – in 6 sections – can also be downloaded:

From Box: https://ucsf.box.com/s/uqzzpsza4w86x2rmulvyua0bufylpsgi

From Dropbox: https://www.dropbox.com/sh/9se9699agk5s5ck/AAAXQKMeELK728MoCzm-Oifua?dl=0

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In order to ensure that we have the best chance of getting the report published in time for the HLM, The Lancet have requested that we formally submit by **April 27th**. I fully appreciate that this means everyone has a short time to review and respond. Nonetheless, we would be grateful for your comments/edits by **April 24** if possible.

In particular, please review the executive summary, in particular the 'vision statement' and the more concise recommendations (5 rather than 10), which align with the HLM 'key asks'.

Eric and I welcome your feedback via email. If possible, share any substantive recommendations/comments in the email, rather than track changes to the documents, to ensure we don't miss any major comments. However, feel free to use track changes if that is your preference. I can also be available most of next week (April 23-26) by 'phone or Skype to discuss questions/clarifications/concerns.

Finally, several of you partnered with non-commissioners to complete writing/analytic products – If you feel these colleagues should be considered as co-authors on the publication, *and you haven't already reached to me to discuss*, please let me know.

Thank you all for your continued support and collaboration in this effort and the important roles you each play in 'building a TB-free world.'

Best regards,

Mike

Lancet Commission on TB, Secretariat

From: "Reid, Michael" (b)(6)
Date: Sunday, April 15, 2018 at 06:22
To: Eric Goosby (b)(6)
Subject: Re: Lancet Commission Conf calls - week of April 16-20

Dear Commissioners,

Apologies for the delay in failing to circle back on this email from David sooner. Unfortunately, we were unable to find any date/time that worked for more than 2 or 3 people. With this in mind, Eric and I have decided to forgo a conference call for all Commissioners.

We are aiming to formally submit a revised draft of the Commission, as well as responses to all the external reviewers' comments, to The Lancet by **April 27**. In advance of that date, I intend to share a pre-submission draft of the full report to all Commissioners this coming Wed/Thurs [**April 18/19**]. I kindly ask that you all take time to review and make edits/comments – sending back any comments/edits no later than April 23.

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I can personally be available for one-on-one calls on April 23 and 24 for anyone that wants to give feedback over the 'phone. Please email me individually if you would like to connect on either day.

Thanks for all of your input, particularly those of you that have been re-writing or reviewing subsections of the report over the last 2 weeks.

Kind regards,

Mike Reid
(on behalf of the Commission Secretariat)

From: David McKey (b)(6)
Date: Wednesday, April 4, 2018 at 09:52
To: David McKey (b)(6)
Cc: "Reid, Michael" (b)(6)
Subject: Lancet Commission Conf calls - week of April 16-20

Dear Commissioners,
As a follow-up to our recent meeting in New Delhi, it was agreed that we would arrange for two conference calls the week of April 16-20 to give an update on the progress and discuss any last minute details before the report is submitted the end of April.

Please find the link for a doodle poll indicating possible times for a call. Please select the two dates and times that work the best for you.
(b)(6)

If you plan on submitting expenses for reimbursement, please send as soon as possible. As an FYI, I will be out on holiday 10-22 April. Expenses not received before 6 April will be submitted upon my return to the office.

And, attached is the picture from our meeting.

Thank you,
David

David McKey

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Office of the UN Secretary-General's Special Envoy on Tuberculosis
Global Health Delivery & Diplomacy
Institute for Global Health Sciences | UCSF
550 16th Street | 3rd Floor | Box 1224
San Francisco, California 94158

(b)(6)
(b)(6)

UCSF Institute for Global Health Sciences

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From: Robin Nandy
Sent: Wed, 25 Apr 2018 21:31:55 +0000
To: Steven Johnson;Fauci, Anthony (NIH/NIAID) [E]
Subject: RE: enjoyed our chat -- hopefully more to come

Dear Steven

It was great to meet you and participate in a panel with you. A true honour.

I will be very interested to learn more about your new project. I am sure you are aware of the work of Hans Rosling and Gapminder but just on the off chance that you are not, here is the web link.

https://www.gapminder.org/

I am sure you will find some pearls there. Please do keep in touch.

Regards

Robin

Dr. Robin Nandy
Principal Advisor & Chief of Immunizations
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From: Steven Johnson [mailto:stevenberlinjohnson@gmail.com]
Sent: Wednesday, April 25, 2018 9:51 AM
To: Robin Nandy <rnandy@unicef.org>; afauci@niaid.nih.gov
Subject: enjoyed our chat -- hopefully more to come

Tony, Robin -- I just wanted to say how much I enjoyed our chat at the Metropolitan Club on Monday night. Was a real honor being on stage with both of you!

I'd like to be back in touch with you both later this year -- one of my upcoming projects (besides the cholera musical!) is a book and TV series about the last hundred years of global life expectancy gains. (The plan is to release the book/show in 2020, the centennial of the end of the

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spanish flu.) The goal is to explain/celebrate just how we have managed to achieve such extraordinary increases in average lifespan, and speculate on the tools/institutions we are going to need to continue those trends over the next century....

For obvious reasons, you would both be invaluable sources of wisdom and experience for this project. So I will be back in touch when we start working on it in earnest...

best,

Steven

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From: Billet, Courtney (NIH/NIAID) [E]
Sent: Wed, 25 Apr 2018 17:53:54 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Folkers, Greg (NIH/NIAID) [E];Conrad, Patricia (NIH/NIAID) [E];Stover, Kathy (NIH/NIAID) [E]
Subject: ASF: For your review, MA re: P. falciparum genetic makeup
Attachments: MA Malaria genes Science_3.docx, NSWB Clearance Sheet Essential Malaria Genes.doc, malaria genes science adams.pdf

Ready for your review. This is a media availability describing the genetic make-up of the *Plasmodium falciparum* malaria parasite. This will appear in Science on May 4.

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DRAFT
EMBARGOED FOR RELEASE
Thurs., Month XX, 2018
2 p.m. Eastern Time

MEDIA AVAILABILITY

Essential Malaria Parasite Genes Revealed
NIAID-funded Research Could Aid Antimalarial Drug Development

WHAT:
Researchers have exploited a quirk in the genetic make-up of the deadly malaria parasite, *Plasmodium falciparum*, to create 38,000 mutant strains and then determine which of the organism's genes are essential to its growth and survival. *P. falciparum* is responsible for about half of all malaria cases and 90 percent of all malaria deaths. New information about the parasite's critical gene repertoire could help investigators prioritize targets for future antimalarial drug development.

The international research team led by John H. Adams, Ph.D., of the University of South Florida, was supported by the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health. The study appears in the Month, Day issue of *Science*.

The complete genetic sequence of *P. falciparum* was determined more than a decade ago, but the functions of most of its genes remain unknown, and until now only a few hundred mutant strains had been created in the lab. The difficulties in manipulating *P. falciparum* stem in part from the extremely high percentage of adenine or thymine (two of the four chemical building blocks that make up DNA) in its genome. Standard methods for creating mutants rely on more variation in gene sequences and so do not work on *P. falciparum*. In the new research, Dr. Adams and his colleagues created mutated versions of nearly all the parasite's 6,000 genes with a technique that preferentially targets areas rich in adenine and thymine, thus exploiting the very feature that had foiled previous attempts at genetic manipulation.

The team used computational analysis to distinguish non-essential genes (those that could be mutated) from essential, non-mutable ones. About 2,600 were identified as indispensable for growth and survival during the parasite's asexual, blood stage. These included ones associated with *P. falciparum*'s ability to resist antimalaria drugs, highlighting them as high-priority targets for new or improved antimalarial compounds, the researchers note.

ARTICLE:
M Zhang *et al.* Uncovering the essential genes of the human malaria parasite *Plasmodium falciparum* by saturation mutagenesis. *Science* DOI:10.1126/science.xxxxxxxx (2018).

WHO:
NIAID Director Anthony S. Fauci, M.D., is available to discuss this research.
Deidre Joy, Ph.D., of NIAID's Parasitology and International Programs Branch, is also available.

CONTACT:

AMERICAN OVERSIGHT

To schedule interviews, please contact Anne A. Oplinger, (301) 402-1663, aoplinger@niaid.nih.gov.

This research was supported, in part, by NIAID grants R01 AI094973, R01 AI117017 and F32 AI112271.

NIAID conducts and supports research—at NIH, throughout the United States, and worldwide—to study the causes of infectious and immune-mediated diseases, and to develop better means of preventing, diagnosing and treating these illnesses. News releases, fact sheets and other NIAID-related materials are available on the NIAID website.

About the National Institutes of Health (NIH): NIH, the nation's medical research agency, includes 27 Institutes and Centers and is a component of the U.S. Department of Health and Human Services. NIH is the primary federal agency conducting and supporting basic, clinical, and translational medical research, and is investigating the causes, treatments, and cures for both common and rare diseases. For more information about NIH and its programs, visit http://www.nih.gov/.

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NIAID News and Science Writing Branch (NSWB)
Clearance Form

__ News Release __x_Media Availability __Statement __NIH Record
__Other (specify):

Title: **Essential Malaria Parasite Genes Revealed**

Audience: Press/Public
Distribution Date/ Embargo Date: Not yet known
NSWB Contact/Writer: Anne Oplinger
Comments: To HHS on Mon. April 23

DATE	NAME
_4/18/18_	Scientific Reviewers: Deirdre Joy, Program Officer, DMID
_N/A____	Branch/Program/Lab Chief:
_OK per BUGS	Division Director:
_N/A__	Budget Officer (as needed)
__N/A_	Beat Supervisor:
_4/18/18	Kathy Stover, NSWB Branch Chief
4/18/18	Courtney Billet, OCGR Director
4/19/18____	Laurie Doepel, Special Assistant to the Director
4/19/18____	Carole Hudgings, Sr. Advisor to the Deputy Director
4/19/18____	Greg Folkers, NIAID Chief of Staff
_____	Hugh Auchincloss, NIAID Deputy Director
_____	Anthony S. Fauci, NIAID Director
4/19/18____	Other Reviewers: H. Marston

Revised: 8/14/2015

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RESEARCH ARTICLE SUMMARY

HEAD SUBJECT

Uncovering the essential genome of the human malaria parasite *Plasmodium falciparum* by saturation mutagenesis

Min Zhang[1†], Chengqi Wang[1†], Thomas D. Otto[2‡], Jenna Oberstaller[1], Xiangyun Liao[1], Swamy R. Adapa[1], Kenneth Udenze[1], Iraad F. Bronner[2], Deborah Cassandra[1], Matthew Mayho[2], Jacqueline Brown[2], Suzanne Li[1], Justin Swanson[1], Julian C. Rayner[2*], Rays H. Y. Jiang[1*], John H. Adams[1*]

INTRODUCTION: Malaria remains a devastating global parasitic disease, with the majority of malaria deaths caused by the highly virulent *Plasmodium falciparum*. The extreme AT-bias of the *P. falciparum* genome has hampered genetic studies through targeted approaches such as homologous recombination or CRISPR-Cas9, and only a few hundred *P. falciparum* mutants have been experimentally generated in the past decades. In this study, we have used high throughput *piggyBac* transposon insertional mutagenesis and Quantitative Insertion Site Sequencing (QIseq) to reach saturation-level mutagenesis of this parasite.

RATIONALE: Our study exploits the AT-richness of *P. falciparum* genome, which provides numerous *piggyBac* transposon insertion targets within both gene coding and non-coding flanking sequences, to generate over 38,000 *P. falciparum* mutants. At this level of mutagenesis, we could distinguish essential genes as non-mutable and dispensable genes as mutable. Subsequently, we identified 2,680 genes essential for *in vitro* asexual blood-stage growth.

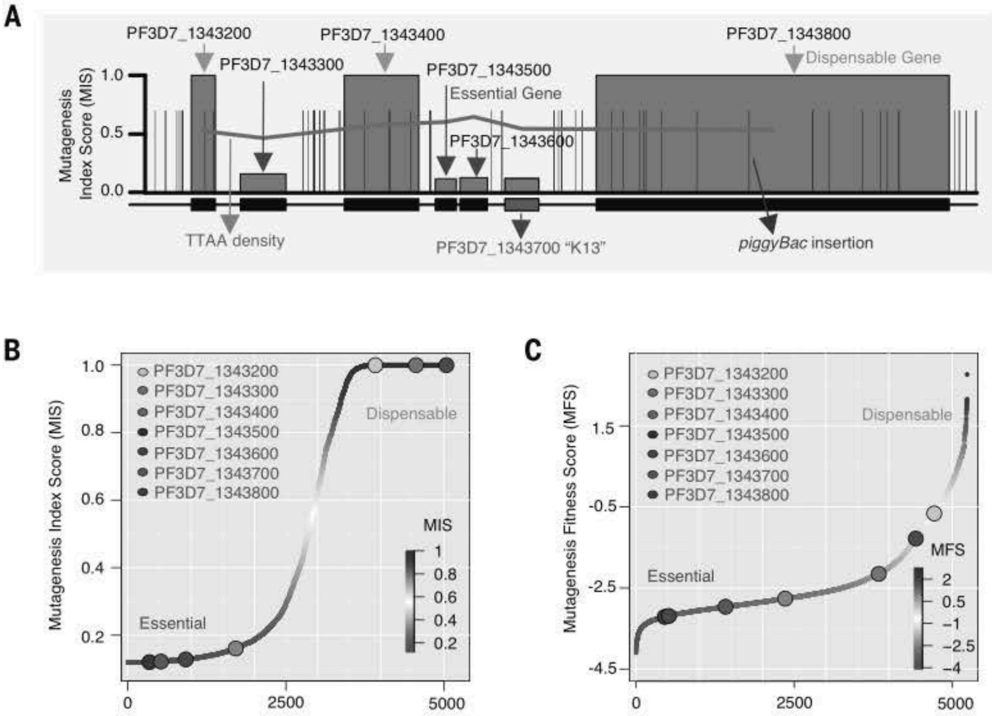
RESULTS: We calculated Mutagenesis Index Scores (MIS) and Mutagenesis Fitness Scores (MFS) to functionally define the relative fitness cost of disruption for 5,399 genes. A competitive growth phenotype screen confirmed that MIS and MFS were predictive of the fitness cost for *in vitro* asexual growth. Genes predicted to be essential included genes implicated in drug resistance, such as the "*K13*" Kelch propeller, *mdr* and *dhfr-ts*, as well as targets considered to be high-value for drugs development such as *pkg*, and *cdpk5*. The screen revealed essential genes that are specific to human *Plasmodium* parasites but absent from rodent-infective species, such as lipid metabolic genes that may be crucial to transmission commitment in human infections. MIS and MFS profiling provides a clear ranking of the relative essentiality of gene ontology (GO) functions in *P. falciparum*. GO pathways associated with translation, RNA metabolism, and cell cycle control are more essential, whereas genes associated with protein phosphorylation, virulence factors, and transcription are more likely to be dispensable. Finally, we confirm that the proteasome-degradation pathway is a high-value druggable target based on its high ratio of essential:dispensable genes, and by functionally confirming its link to the mode of action of artemisinin, the current front-line antimalarial.

CONCLUSION: Saturation-scale mutagenesis allows prioritization of intervention targets in the genome of the most important cause of malaria. The identification of over 2,680 essential genes, including ~1000 *Plasmodium*-conserved essential genes, will be valuable for antimalarial therapeutic research. ■

ON OUR WEB SITE

Read the full article at http://dx.doi.org/10.1126/science.aap7847

The list of author affiliations is available in the full article online.
*Corresponding author. E-mail: xxxxxxxxx@xxxx.au;
Cite this article as X. X. Xxxxxxx *et al.*, *Science* XXX, 0000000 (2014). DOI: 10.1126/science.xxxxxxxxx



Saturation-scale mutagenesis of *Plasmodium falciparum* reveals genes essential and dispensable for asexual blood-stage development. (**A**) A high-resolution map of a ~50 KB region of chromosome 13 depicts an essential gene cluster, including K13, that lack insertions in the coding sequence (CDS) but is flanked by dispensable genes with multiple CDS-disrupting insertions. (**B**) The Mutagenesis Index Score (MIS) rates the potential mutability of *P. falciparum* genes based on the number of recovered CDS insertions relative to the potential number that could be recovered by large-scale mutagenesis. (**C**) The Mutagenesis Fitness Score (MFS) rates the relative fitness of *P. falciparum* genes based on QIseq scores of transposon insertion sites in each gene.

PHOTO: CREDIT GOES HERE AS SHOWN; CREDIT GOES HERE AS SHOWN

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HHS-NIAID-20-0593-A-001564

Science AAAS

Submitted Manuscript: Confidential

Uncovering the essential genes of the human malaria parasite *Plasmodium falciparum* by saturation mutagenesis

Authors: Min Zhang[1†], Chengqi Wang[1†], Thomas D. Otto[2‡], Jenna Oberstaller[1], Xiangyun Liao[1], Swamy R. Adapa[1], Kenneth Udenze[1], Iraad F. Bronner[2], Deborah Cassandra[1], Matthew Mayho[2], Jacqueline Brown[2], Suzanne Li[1], Justin Swanson[1], Julian C. Rayner[2*], Rays H. Y. Jiang[1*], John H. Adams[1*]

[1]Center for Global Health and Infectious Diseases, Department of Global Health, University of South Florida, 3720 Spectrum Blvd, Suite 404, Tampa, Florida 33612.

[2]Malaria Programme, Wellcome Trust Sanger Institute, Genome Campus Hinxton Cambridgeshire, CB10 1SA United Kingdom.

†M.Z. and C.W. contributed equally to this work.

‡Current address: Centre of Immunobiology, Institute of Infection, Immunity & Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom

* Corresponding authors. Email: jadams3@health.usf.edu, jiang2@health.usf.edu & jr9@sanger.ac.uk.

Severe malaria is caused by the apicomplexan parasite *Plasmodium falciparum.* Despite decades of research the unique biology of these parasites has made it challenging to establish high throughput genetic approaches to identify and prioritize therapeutic targets. Using transposon mutagenesis of *P. falciparum* in an approach that exploited its AT-rich genome we generated >38,000 mutants, saturating the genome and defining mutability and fitness costs for >87% of genes. Of 5,399 genes our study defined 2,680 genes essential for optimal growth of asexual blood-stages *in vitro*. These essential genes are associated with drug resistance, represent leading vaccine candidates, and include ~1000 *Plasmodium*-conserved conserved proteins of unknown function. We validated this approach by testing proteasome pathways for individual mutants associated with artemisinin sensitivity.

One Sentence Summary: (150 characters) Saturation-scale mutagenesis of *Plasmodium falciparum* reveals a core set of genes essential for asexual blood-stage growth *in vitro*.

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Malaria caused by *Plasmodium falciparum* remains an insidious global health problem, with hundreds of thousands of deaths each year. Recently there have been significant reductions in disease intensity, in part through concerted recent use of artemisinin-combination therapies, but these gains are now threatened by emerging ACT treatment failures spreading across South East Asia (*1, 2*). If ACT resistance reaches Africa, a devastating rebound of disease is expected, as occurred with chloroquine resistance in the 1970s. The development of new antimalarial therapies, and identification and prioritization of new targets, is a priority. More than a decade after the completion of the *P. falciparum* genome, a significant fraction of its genome still lacks functional annotation (*3*). Although CRISPR-Cas9 and other targeted endonucleases will accelerate functional genomics studies (*4-6*), their usefulness for genome-scale applications is restricted by the absence of non-homologous end joining in *Plasmodium* parasites, and by the extreme AT-richness of the *P. falciparum* genome which reduces gRNA target site abundance. Therefore, a critical need remains for large-scale genetic analysis to systematically identify essential genes and prioritize parasite metabolic pathways for drug discovery (*7*).

Large-scale genetic screening methods in model organisms rely on efficient scalable methods for genome engineering. Transposon mutagenesis using the *piggyBac* transposon, which preferentially inserts at the tetranucleotide target sequence TTAA, has been used to carry out whole-genome loss-of-function screens in many organisms (*8-11*). The highly skewed nucleotide composition of the *P. falciparum* genome, with >81% AT content, is an advantage for the application of *piggyBac* mutagenesis. The skewed composition results in a high density of TTAA sites, averaging one site per 70 bp over both coding and non-coding regions, in theory allowing systematic and saturation-level mutagenesis of the whole genome. While *piggyBac* mutagenesis has been developed and optimized for *P. falciparum,* and previously used for small-scale phenotypic screens and functional characterization of loss-of-function mutants, it has not been used for large-scale screening (*12-16*). To scale up *piggyBac* mutagenesis in *P. falciparum*, we developed high-throughput transfection mutagenesis

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methods that mostly create a single insertion per genome, and combined them with an Illumina-based sequencing method for identifying transposon insertion sites (*17, 18*). This approach, known as Quantitative Insertion-site Sequencing (QIseq), thus allowed whole-genome experimental mutagenesis analysis of *P. falciparum* (fig. S1, A and B).

Achieving saturation-level mutagenesis

In a preliminary study we carried out large-scale transfections followed by short-term *in vitro* growth of mixed pools of drug-selected *P. falciparum* parasites and identified insertion sites by QIseq. This pilot identified a total of 3,651 insertions across the *P. falciparum* genome (table S1)(*17, 19*). Based on the density and distribution of those insertions modeled by a Negative Binomial Distribution, we predicted that recovery of ≥33,000 insertions would be sufficient to achieve saturation-level mutagenesis in the compact genome of the malaria parasite (fig. S1C-F); this number is sufficient for there to be a high probability that multiple transposon insertions would occur within the CDS of every protein-coding gene larger than 500 bp. We therefore scaled our transfection methods to achieve this number of insertions. Transfected parasite populations were drug-selected briefly to isolate only mutant parasites with integrated *piggyBac* elements carrying the drug resistance gene *hdhfr*, before insertion-site locations were identified by QIseq. Computational analyses were used to verify the sequence reads of the QIseq libraries and the consistency of the raw data (fig. S2A-F); nearly all QIseq-defined insertions occurred at the characteristic TTAA target sequence. All insertions sites that were not flanked by the consensus TTAA (2.49%) insertion sites were removed from subsequent analysis (fig. S2A). Previously validated individual *piggyBac* mutant clones were included in each QIseq run to act as a control for the accuracy and sensitivity of each insertion-site identification run (fig. S3A-C and table S2).

The saturation mutagenesis approach identified ~38,000 independent *piggyBac* insertions at distinct TTAA target sites, covering 5,399 nuclear protein-coding genes across all 14 chromosomes of the *P. falciparum* genome (Fig. 1A and table S3). These randomly distributed insertions exceeded those predicted to be required to achieve saturation-level mutagenesis (Fig. 1A), but there were numerous genes and regions encompassing several genes that had significantly fewer insertions than would be expected based purely on the distribution of

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TTAA-sites across the genome (Fig. 1B, C and D). As well as discrepancies in non-random spatial distribution, coding regions overall lacked insertions compared with intergenic regions ($p < 2.2e-16$, Fisher test) (Fig. 1D). A more detailed analysis of *piggyBac* insertion density within transcriptional units revealed that insertions in CDS were threefold less common than in flanking intergenic regions, and even within intergenic regions, insertion-site density decreased with proximity to CDS (Fig. 1E). This significant bias towards recovering intergenic insertions in surviving blood-stage parasites, with many CDS having no insertions, are indications that genes lacking insertions are lethal when disrupted by *piggyBac* insertion.

Defining gene dispensability using mutagenesis index scores

While there was a bias against insertions in CDSs, 2,042 genes were disrupted by at least one *piggyBac* insertion (~38% of genes in the *P. falciparum* genome) (Fig. 1F). The remainder, 3,357 genes (~62% of the genome) had no insertions in their CDSs, and some of these were also completely devoid of insertions in the surrounding intergenic regions (2.9% of genes) (Fig. 1C, fig. S4A and table S4). In the 2,042 mutable genes, insertion sites were distributed uniformly along the gene body CDSs, indicating all disruptions have equivalent consequences for the disrupted gene (Fig. 1E). The uniform distribution of transposon insertions throughout the genome is independent from chromatin structure and gene locations (fig. S4B). We therefore reasoned that the recovery of one or more insertions within the CDS of disrupted genes was an indicator of gene dispensability for *in vitro* asexual blood-stage growth. Therefore, mutable genes are subsequently referred to as dispensable, while the absence of any insertions in the CDS could be considered an indicator that disruptions are lethal, and subsequently these genes are referred to as essential. However, the essential classification of 677 genes without insertions, which are small or with lower TTAA, is tentative, since they are below the average distance between recovered insertions (<613 nt)(table S5). To quantify the evidence for dispensability or essentiality of each gene we developed a mutagenesis index score (MIS) based on the number of identified *piggyBac* insertions relative to the number of available TTAA target sites within that gene (Fig. 2A and table S5). Genes with higher MIS (on a scale of 0 to 1) were considered to have a higher possibility of dispensability, while those with

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lower MIS were considered to have a higher possibility of essentiality. The 2000 genes with highest and lowest MIS represent mutability characterization with the strongest confidence.

Biological processes identified as dispensable and essential

Mutants in the lowest MIS quartile were enriched in core metabolic processes shared across eukaryotes, which would be predicted to be essential. Mutants in the highest MIS quartile were enriched in parasite-specific multi-gene families that interact directly with the host, and which are known to be partially functionally overlapping and contain many redundant genes (Fig. 2B). It is important to note that this screen was carried out using *in vitro* cultured parasites, and many of these *in vitro* dispensable genes, such as those for antigenic variation and cytoadherence, have greater importance *in vivo* in human infections (fig. S5). The dispensability of parasite processes involved in host-parasite interactions contrasted with the essentiality of internal parasite processes, such as those associated with RNA metabolism (Fig. 2C-D, fig. S6 and table S6). *RNase II*, a gene implicated in a post-transcriptional regulatory mechanism relevant to severe malaria, is an example with a low MIS score, consistent with previous reports of the locus being refractory to disruption (*20*). Other genes involved in general RNA metabolism that have low MIS include both widely-conserved RNA-binding proteins (such as *PABP*) and apicomplexan-specific RNA-binding proteins likely to have more specific, mostly unidentified, regulatory targets. The *P. falciparum* genome has an abundance of RNA-binding proteins that are largely functionally uncharacterized and our analysis suggests that many of these genes are likely to be essential.

MIS therefore correlates with what is known broadly about the importance or redundancy of metabolic pathways. To further validate that MIS is a good predictor of essentiality, we analyzed genes that have been the focus of drug or vaccine development. Genes strongly predicted to be essential based on MIS included the "*K13*" Kelch propeller implicated in artemisinin resistance, as well as other genes implicated in drug resistance, such as *DHFR-TS, MDR* and *AAC2,* involved in pyrimethamine, mefloquine and atovaquone reistance, respectively. Other genes classified as essential based on MIS included ones considered high priority blood-

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stage drug targets such as *PKG*, and *CDPK5*. By contrast, most blood-stage vaccine candidates were dispensable, with the notable exception of *RH5*. Not unexpectedly, sporozoite vaccine candidates CSP and TRAP, which are essential for sporozoite development in mosquitoes but are not required in blood-stage development (*21*), had high MIS. In contrast, the gene for pore-forming protein Cell Traversal of Ookinetes and Sporozoites (CelTOS) had low MIS. CelTOS is important for these parasite migratory phases and is emerging as a pre-erythrocytic-transmission blocking vaccine candidate; its low MIS indicates it may also have an essential function in blood-stage infections, making it a potential multi-stage vaccine target. MIS analysis also revealed likely essential genes that are specific to human *Plasmodium* parasites but absent from rodent-infective species, such as the lipid metabolic genes (PCD, PMT) that are crucial in the development of mosquito-transmissible sexual-stages in *P. falciparum* (*22, 23*).

Mutant growth fitness as a measure of gene dispensability

We previously developed a phenotyping method in which a pool of *piggyBac* mutants were grown as mixed-mutant pools over multiple generations, and the number of reads for each *piggyBac* insertion site quantified using next-gen sequencing to measure parasite growth rates (*17*). We adapted this method to generate a second quantitative measure of gene dispensability independent from MIS, by comparing the normalized number of reads from each insertion site to the total pool of reads across all *P. falciparum* mutants in the saturation mutagenesis screen (Fig. 2E and table S5). This mutagenesis fitness score (MFS) serves as a proxy for mutant growth fitness. MFS scores strongly correlated with MIS scores, despite the fact that they are independent of each other (Fig. 2F). The MFS of mutable genes was high, in keeping with the MIS prediction of dispensability, while predicted essential non-mutable genes had a low MFS (Fig. 2G). Intermediate MIS and MFS scores were weakly correlated, in keeping with the lower confidence that we can place on the essentiality or dispensability of genes with these intermediate scores.

A competitive *in vitro* growth screen (*17*) was used to phenotype four separate pools of mixed-mutant populations to provide additional validation of the correlation between MIS and a gene's importance for growth

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fitness. Mutable genes with high MIS had low or minimal fitness cost were competitive growth 'winners', whereas growth fitness 'losers' had relatively low MIS (Fig. 3A and table S7). Relatively few mutations had little or no fitness cost, as measured by MFS, resulting in a disproportionate number of mutant 'losers' (Fig. 3B). Overall both the MIS and MFS were predictive of the fitness cost for *in vitro* asexual growth (Fig. 3C), and between these metrics we were able to predict separate 5,399 genes in the *P. falciparum* genome into non-mutable and mutable categories (Fig. 3D and E).

Association of essentiality with genome structure and transcription patterns

Dispensable and essential genes rarely occurred as single isolated genes, but rather occurred as multi-gene clusters reminiscent of conserved syntenic blocks (Fig. 1A, C and fig. S4A)(*24*). We therefore assessed the relationship between evolutionary conservation of genome structure and essentiality/dispensability by using previously defined syntenic relationships between *Plasmodium spp*. (*25*). Syntenic genes indeed had lower MIS, suggesting conserved essential functions across *Plasmodium* spp.; conversely, non-syntenic genes were more likely to be dispensable (Fig. 4A). Mapping chromosomal regions with synteny breaks, which typically harbor gene duplications and paralogs, showed patterns of clustering of essential and dispensable genes in syntenic and non-syntenic chromosomal regions, as seen in examples for chromosomes 13 and 10 (Fig. 4B-E).

Transcription metrics for each gene based on maximum FPKM values (*26*) were examined for correlations with essentiality and dispensability (Fig. 5A). This analysis showed that essential genes are expressed at significantly higher levels throughout both the asexual and sexual life cycle phases, indicating they may have critical functional roles throughout both the mosquito and human stages and therefore represent potential multi-stage drug targets (Figs. 5B and C). Within the intraerythrocytic cycle, genes with peak expression during the trophozoite stage were more likely to be essential than those expressed during other stages (Fig. 5C). During the trophozoite stage, *P. falciparum* must acquire nutrients from the host and remodel the infected erythrocyte to avoid host defenses, such as clearance by the spleen. In contrast, dispensable processes were enriched at either end of intraerythrocytic development, reflecting parasite stages with a greater need for functional redundancy, such as those involved in host interaction (e.g., erythrocyte invasion, antigenic

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variation), and are hence more likely to undergo duplication/paralog evolution. Not unexpectedly, about 41% of the dispensable genes are predominantly expressed in sexual stages (Fig. 5C). These genes may be essential for transmission to or from the mosquito, but do not appear to have an essential function for asexual blood-stage development. The high coverage of sexual-stage genes in this screen emphasizes the potential for *piggyBac*-based screens for identifying sexual phenotypes.

Evolutionary conservation of apicomplexan gene essentiality

We compared the data from our genome-wide saturation screen with data from large-scale genome sequencing studies of *P. falciparum*, as well as data from recent large-scale mutagenesis studies of apicomplexan genomes (*6, 18, 27-29*) to evaluate the conservation of gene essentiality across the organisms' lineages. Essential *P. falciparum* genes were more highly conserved across *Plasmodium spp*. (Fig. 5D and fig. S8A), are less likely to have a paralog (Fig. 5E and fig. S8B), and encode genes with less genetic variation among *P. falciparum* clinical isolates (Fig. 5F and fig. S8C). Of the *P. falciparum* genes studied here, 2,083 and 1,998 have orthologs in the more distantly-related parasites *Toxoplasma gondii* (*6*) and *P. berghei* (*29*), respectively. Overall, there was a strong correlation in gene function between species, particularly with genes predicted as essential (Fig. 5G and H and fig. S8D and E). Genes that are dispensable in *P. falciparum* were also more likely to be dispensable in *P. berghei*. By contrast, the correlation between the dispensable genes of *P. falciparum* and *T. gondii* was weaker, indicating that genus-specific gene functions are enriched for dispensability. Overall, these comparisons show that while phenotype classifications from the large-scale mutagenesis screens in apicomplexan species are also broadly predictive of the *P. falciparum* phenotype classifications, the closer evolutionary relationship of *P. berghei* provides a higher level of sensitivity and specificity for the orthologs that could be disrupted (Fig. 5I and fig. S8F).

Consistent with this assessment that non-mutable genes represented core essential functions in *P. falciparum*, GO enrichment analysis indicated genes with functions associated with translation, RNA metabolism, and cell cycle control were more likely to be essential, whereas genes associated with protein phosphorylation, virulence factors, and transcription were more likely to be dispensable (Fig. 6A). Comparing

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the relative number of essential, versus dispensable, genes within specific GO biological processes, molecular functions, and cellular components provided a ranking of the essentiality of potential druggable targets and pathways (Figs. 6A-C, figs. S9A-C and table S8). For example, nearly all genes annotated as being involved in ubiquitin-dependent degradation processes were experimentally defined as essential, while individual genes linked to microtubule motility and antigenic variation are experimentally defined as dispensable based on MIS. RNA metabolism and translation-related processes are also highly essential, supporting emerging evidence for the importance of post-transcriptional and translational control (*30*).

Essentiality of the proteasome-degradation pathway

Recent genetic analysis of artemisinin-combination therapy resistance has linked drug resistance to cellular stress-response mechanisms involving the *P. falciparum* ubiquitin/proteasome system (*31, 32*). Genes of the proteasome-degradation pathway were well represented in the *piggyBac* insertion set, and 54 of the 72 genes could be classified as essential genes based on our data (fig. S10A), further strengthening the priority of the proteasome degradation pathway as a high-value druggable target (*33*). We validated this link between artemisinin sensitivity and proteasome inhibition sensitivity using a set of single-insertion *piggyBac* mutants previously defined by chemogenomic profiling to be part of an artemisinin-sensitivity cluster, including a mutant of the K13 Kelch propeller gene (*16, 34*). We found ten-fold increased sensitivity to the proteasome inhibitor Bortezomib in mutants of the artemisinin-sensitivity cluster; significantly different relative to wild-type NF54 or mutants with distinct chemogenomic profiles (fig. S10B-E), providing additional support for the association between the ART mechanism of action and proteasome function.

Summary

In conclusion, our genome-wide saturation mutagenesis screen in the major human pathogen, *P. falciparum* has defined genes essential for parasite survival during the blood stage and provided critical functional data for prioritizing high-value drug targets and pathways. The complete characterization of the essential genome with high-throughput saturation mutagenesis will help open new frontiers for antimalarial

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therapeutic research. The methodology also opens the way for new systematic functional screens for other phenotypes, such as transmission and cytoadherence.

Materials and Methods

Parasite culture

All *P. falciparum* parasite NF54 and *piggyBac* mutants were cultured in complete RPMI 1640 with 5% hematocrit (medium containing 0.5% Albumax II, 0.25% sodium bicarbonate and 0.01 mg/ml gentamicin). All parasite cultures were maintained by standard methods (*35*).

piggyBac Transfection (35)

High-efficiency transfection (96-well plate method) was carried out on NF54 schizonts purified by magnetic column (MiltyenyiBiotec, CS column) using a transposon plasmid (pLBacII-HDH, containing selection marker human *dhfr*) and a transposase-expressing helper plasmid (pDCTH)(*19*). RBCs were first loaded with plasmid DNA by electroporation using Gene PulserXCell+CE Module (BioRad). A total of 10 million schizonts, 1200 µg plasmid DNA and 600 µg helper plasmid DNA were used per 96-well plate to achieve maximal transfection efficiency. The drug WR99210 (final concentration 2.5 nM) was added to each plate for selecting transfected parasites, using a robot (Integra VIAFLO 96) to change media with drug every day for 5 days. Transfections were performed in batches over the course of 4 to 6 weeks. Each 96-well plate was cultured in triplicate and cryopreserved in duplicate. One hundred 96-well plates were used to generate the large pools for sequencing (fig. S1A).

For maximum recovery of unique mutants, the transfected culture was distributed into 96-well plates by robot (200µl each well) and grown under two rounds of drug selection. Wells were screened for parasites by PCR and Giemsa-stained thin blood films. >50% of wells became parasite-positive within three weeks. Each

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positive well with ≥9 unique mutants (one well = one "Mixed Population", or MP) was cryopreserved in duplicate (two 96 well plates). Additional 96-well plates were used to generate the pools that were then cultured in a T75 flask and harvested after two cycles growth by standard methods for genomic DNA isolation for QIseq (*17*). Although mutants grow at different rates, the pools can be expanded for at least 12 asexual life cycle generations ("cycles") without significant loss in detectable diversity. Importantly, each mutant pool can be regenerated and cloned from their original MPs.

Datasets of reference genomes, transcriptome data and epigenetics data

The NF54 reference genome can be downloaded from (ftp://ftp.sanger.ac.uk/pub/project/pathogens/Plasmodium/falciparum/NF54/Assembly/V1_morphed/). The QIseq sequencing mapping followed previously published methods (*17*). Ortholog counts, paralog counts, and non-synonymous/synonymous (NonSyn/Syn) ratios can be downloaded from PlasmoDB (http://plasmodb.org/plasmo/) (*36*). Previously published RNA-seq expression data (*26, 37*) are used in computational analysis. Formaldehyde-Assisted Isolation of Regulatory Elements (FAIRE-seq) data of seven time points (0, 6, 12, 18, 24, 30 and 36 hour) were used to measure chromatin accessibility (*38*). The motif information of transcription factor ApiAP2 binding sites is available in previously published work (*39*). The genome synteny across five *Plasmodium* species is available in published work (*25*).

Identification of > 38,000 mutants carrying piggyBac insertion

The QIseq sequencing generated a total of 3,301,112 raw reads. First, all the raw reads were filtered by the criteria of matching expected transposon target site 'TTAA'. This filtering step yielded 2,042,147 reads, which is >6-fold genome coverage. The insertion site QIseq signal was calculated as the reads count of the filtered QIseq reads. To compare the insertion signals between different QIseq runs, we normalized for each insertion *i* as

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$$S_i = \frac{c_i}{\sum_{j \in R} c_j} \times 250000 \quad (1)$$

, where S_j represents the signal of the insertions i with reads counts c_i in run R. All the *piggyBac* insertions with reads in both 5′ and 3′ ends were treated as true insertions by using an extremely high level of accuracy in target site (target motif TTAA ~ 99%). To identify true insertions with reads only in single 5′ or 3′ ends, we used a method that selected the most accurate signal cutoff based on the ratio of the number of input reads/ the number of output disrupted genes. We computed the number of gene CDSs targeted by the insertions upper cutoff (i.e. more accurate). We noticed that there was a single large and sharp increase in the number of CDSs targeted by the insertions with reads number increasing from cutoff 2.0 to cutoff 2.3 (Fig. S2E, $p < 0.05$ compared with changing another cutoff). The result indicated a large amount of false positive may be included when setting cutoff ≥ 2.3. Therefore, the normalized reads count = 2.3 was set as the lower bound for identifying true insertions.

Mutagenesis saturation computational validation

To evaluate the levels of whole-genome mutagenesis and eventual saturation, we used a computational method based on sampling. First, a specific number of insertions were randomly extracted and the fraction of genome elements targeted were counted. This procedure was repeated 1000 times for each query insertion number in fig. S1C. A log-based curve was observed by plotting the mutational events and the median number of genome elements targeted by insertions. The plateauing of the curve at about 20,000 mutational events indicated that a very small number of new genome elements could be targeted even with very large amount of insertions. Importantly, the real data-based sampling curve was very similar to our independent mathematical predictions of Negative Binomial distributions prior to the start of the saturation mutagenesis experiments (fig. S1D). Together, both our mathematical model and real-data computational sampling show that > 33,000 mutants represent saturation-level mutagenesis of the entire set of protein-encoding genes in the *P. falciparum* genome.

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Sampling methods for accounting for fragment length differences and nucleoside composition bias

For genes in the *P. falciparum* genome, 5′ upstream and 3′ downstream intergenic regions displayed similar TTAA densities (median number of TTAA sites per 100bp were 1.82 and 1.89, respectively). However, they have significantly different region length distributions (median values are 1681bp and 926bp in 5′ and 3′ direction, respectively; $p < 0.001$ Wilcoxon test). Therefore, there are different numbers of genomic TTAA sites in 5′ and 3′ intergenic regions, as an intrinsic feature of the *P. falciparum* genome. To account for this region-length and total-TTAA bias in our QIseq calculations, the TTAA sites in 3′ intergenic regions were sampled based on the TTAA number in 5′ intergenic regions, so that our comparison was based on two identical background target-site distributions. Distribution of insertions and TTAA sites (Fig. 1E) were plotted based on these sampling data.

Mutagenesis index score (MIS) calculation

To quantify the mutability of every protein-coding gene in the *P. falciparum* genome, we first checked the number of *piggyBac* insertions located inside the gene CDS regions. To keep the criteria stringent, the insertions located on the very end (distance from insertion to TSS > 99% of the CDS length) of the CDS were not considered, due to a significantly higher number of insertions at the last 1% of the CDS compared with other regions of the CDS (fig. S2F). We calculated the initial score MIS of gene MSg based on the equation

$$MS_g = \log\left(\frac{N_g+1}{D_g}\right) \qquad (2)$$

where N_g represents the number of insertions on gene g. D_g is the TTAA density of the gene g, which could be represented as the number of TTAA per kb of the CDS. We found that the *MS* could be decomposed into two mixed Gaussian distributions (Fig. S11). We reasoned that dispensable and essential genes exhibit different *MS* distribution. Therefore, a binary variable π_g was used to model whether the gene g is dispensable or not: $\pi_g =$

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1 corresponds to dispensable and vice versa. The probability of observing a MS_g of gene g is mixture of two Gaussian distributions:

$$P(MS_g) = \sum_{\pi_g=1,0} P(\pi_g) N(MS_g | \mu_{\pi_g}, \sigma_{\pi_g}) \qquad \sum_{\pi_g=1,0} P(\pi_g) = 1 \qquad (3)$$

Subsequently, the EM algorithm was implemented to search for the optimized value of parameters. The posterior distribution after the EM optimization procedures could be calculated as:

$$P(\pi_g = 1 | MS_g, \Theta) = \frac{P(\pi_g=1) N(MS_g | \mu_{\pi_g=1}, \sigma_{\pi_g=1})}{\sum_{\pi_g=1,0} P(\pi_g) N(MS_g | \mu_{\pi_g}, \sigma_{\pi_g})} \qquad (4)$$

,where Θ is the parameter space. The MIS was defined as the posterior distribution to be dispensable.

Mutagenesis fitness score (MFS) calculation

For the mutants that were subjected to competitive growth essay, we calculated MFS to represent the comparative growth fitness of a mutant. The QIseq sequencing reads distribution reflects the fitness of unique *piggyBac* mutants in the competitive-growth assays. The start and the end of the growth assay could be represented as $t_0$and t. At the end of the assay, $a_{t,g}^{m,v}$indicateed the relative abundance of each mutant *m,* targeted into the CDS of gene g in sample *v*. Therefore, the abundance of the mutant of the gene g at the end of the assay could be represented as $\sum_{m\epsilon v} a_{t,g}^{m,v}$. The $\sum_{m\epsilon v} a_{t,g}^{m,v}$ was directly proportional to the QIseq reads number $\sum_{m\epsilon v} r_{t,g}^{m,v}$. Here, $r_{t,g}^{m,v}$ represents the normalized reads number of mutant *m* targeting gene g on CDS, which is $\sum_{m\epsilon v} a_{t,g}^{m,v} \sim \sum_{m\epsilon v} r_{t,g}^{m,v}$. The QIseq reads starting with 'TTAA' were used to measure $r_{t,g}^{m,v}$. To normalize different $\sum_{m\epsilon v} r_{t,g}^{m,v}$in different sample *v*, the average value of $\sum_{m\epsilon v} r_{t,g}^{m,v}$ was calculated as

$$\sum_v \sum_m r_{t,g}^{m,v} / \sum_m I(m \text{ on } g) \qquad (5)$$

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Here, $I(\cdot)$ is the indicator function. $\sum_m I(m\ on\ g)$ was the number of mutants targeting gene g in all samples. At the start of the assay t_0, the relative abundance of each mutant equal to the background TTAA density D_g. Finally, the MFS could be calculated as:

$$MFS_g = \log\left(\frac{\sum_v \sum_m r_{t,g}^{m,v} / \sum_m I(m\ on\ g)}{D_g}\right) \quad (6)$$

Therefore, the MFS calculation based on individual mutant abundance is a proxy for competitive growth fitness in *in vitro* blood stage.

GO analysis

All the Gene Ontology (GO) terms were downloaded from (http://plasmodb.org/plasmo/)[27]. For each specific GO term g, the MIS distribution in the term g could be represented as MIS_g. The number of genes in term g is Num_g. We asked whether the genes in the term g are more prone to be dispensable or essential. To answer this question, we compared the median value of MIS_g with sampled data distribution as background. The genes with the same number of Num_g were sampled out and represented as S_g. This procedure was repeated 1000 times and the median MIS of each $S_{g,t}$ ($t \in 1,2,3..1000$) was calculated as $MIS_{S_{g,t}}$. The p-value was calculated as

$$p_g = \begin{cases} \sum_{t=1}^{1000} I\left(MIS_g > MIS_{S_{g,t}}\right)/1000 & (MIS_g > 0.5) \\ \sum_{t=1}^{1000} I\left(MIS_g < MIS_{S_{g,t}}\right)/1000 & (MIS_g < 0.5) \end{cases} \quad (7)$$

Here, $I(\cdot)$is the indicator function.

Half-maximal inhibitory concentration (IC50) estimation

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To calculate IC50, the dose-response data (e.g., drug concentrations $c_1, c_2, \ldots c_n$ and growth inhibition $q_1, q_2, \ldots q_n$) were used to fit a Hill Equation.

$$q = U + \frac{U - D}{1 + 10^{(c - \log C)B}} \tag{8}$$

where c was drug concentrations in logarithmic form. The parameter C was the estimate of IC50. The goodness of fit was calculated from dose-response data and the Hill Equation.

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Acknowledgements

All data and code to understand and assess the conclusions of this research are available in the main text, supplementary materials and deposited into the European Nucleotide Archive. Accession numbers for individual experiments and libraries are listed in table S9.

piggyBac transfection plasmids (MRA911/912) and mutants are deposited with the Malaria Research Reagent and Reference Repository (BEI Resources). *piggyBac* transfection plasmids and mutant parasites may be obtained directly from the authors at the University of South Florida, using a standard academic MTA based upon the UBMTA.

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US Patent 7932088 (April 26, 2011). High Efficiency Transformation of *Plasmodium falciparum* by the Lepidopteran Transposon, *piggyBac*. Inventors: JH Adams, MJ Fraser, Jr., B Balu, DA Shoue. Invention relates to use of *piggyBac* as a tool for genetic manipulation of the Plasmodium genome.

Author contributions.

Transfection and cell culture: M.Z., X.L., K.U., D.C., S.L., J.S.; quantitative insertion-site sequencing: M.Z., I.F.B., M.M., J.B.; computational analysis: C.W., M.Z., T.D.O., J.O., S.R.A., R.H.Y.J.; writing group: M.Z., C.W., T.D.O., J.C.R., R.H.Y.J., J.H.A.; conceived and directed the study: J.C.R., R.H.Y.J., J.H.A.

Supplementary Materials

Figs. S1 to S11

Tables S1 to S9

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Figure legends

Fig. 1. A genome-wide saturation mutagenesis screen for *Plasmodium falciparum.*

(**A**) Chromosomal map displays 38,173 *piggyBac* insertion sites from all mutants evenly distributed throughout the genome. (**B**) High-resolution map of a ~50 KB region of chromosome 13 depicts an essential gene cluster, including K13, flanked by dispensable genes with multiple CDS-disrupting insertions. (**C**) High-resolution map of a ~20 KB region without insertions includes three conserved genes of unknown function (PF3D7_1232700, PF3D7_1232800, PF3D7_1232900) and a putative nucleotidyltransferase (PF3D7_1232600) (see also fig. S5). (**D**) A plot of all *piggyBac* insertions revealed significantly fewer insertions were recovered from exon-intron regions compared to the proportion of available TTAA sites (see also fig. S1D) ($p < 2.2e-16$, Fisher's Exact test). (**E**) Density of *piggyBac* insertion-site distribution revealed threefold fewer insertions recovered in transcriptional regions (blue) than intergenic 5′ (yellow) and 3′ (green) regions, depicted as relative distance upstream and downstream to a gene, respectively. (**F**) This study determined that under ideal culture conditions for asexual blood-stage growth, 38% of genes in the *P. falciparum* genome have mutable CDSs, while 62% of genes have non-mutable CDSs, which includes 12% with tentative classification.

Fig. 2. Identification of dispensable and essential genes through Mutagenesis Index Score (MIS) and Fitness Score (MFS).

(**A**) The Mutagenesis Index Score (MIS) rates the potential mutability of *P. falciparum* genes based on the number of recovered CDS insertions relative to the potential number that could be recovered. Genes known as dispensable or essential are highlighted. (**B**) MIS violin plots of GO processes grouped from lowest to highest dispensability, according to gene functional annotations. (**C**) MIS plots and (**D**) high-resolution chromosome maps highlighting important genes of interest for RNA metabolism [-20kb, +20kb] (see also fig. S4 for MIS plots of other genes of interest. (**E**) The Mutagenesis Fitness Score (MFS) estimates the relative growth fitness

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cost for mutating a gene based on its normalized QIseq sequencing reads distribution. (**F**) MIS has significant correlation to MFS (Pearson's R = 0.67, $p < 2.2e-16$ compared with permutation). (**G**) The first and second MFS quartiles were comprised primarily of non-mutable genes, the 4th quartile was comprised mostly of mutable genes, and 3rd quartile had nearly equal numbers of both.

Fig. 3. Validation of mutagenesis score through phenotype screen.

(**A**) Competitive growth assays of asexual blood-stage growth under ideal *in vitro* culture conditions: phenotypes of four independent mixed-population pools grown for three cycles confirmed losers (left, bottom quantile) and winners (right, top quartile) had significantly different MIS. (**B**) Overall rank-ordered plot of competitive growth phenotypes shows losers and winners. (**C**) Competitive growth 'losers' had significantly lower MIS and MFS, respectively, validating MIS and MFS as predictor of gene essentiality and dispensability. (**D**) Circos plot from outer to inner shows the distribution of all *piggyBac* insertions, MIS (pink indicates MIS < 0.5, while blue is >0.5), CDS insertions, and MFS along each chromosome of *P. falciparum* genome. (**E**) Violin plots indicate non-mutable genes had significantly lower MIS and MFS ('****' represents Wilcoxon $p < 2.2e-16$).

Fig. 4. Chromosomal syntenic breakpoints are enriched in dispensable genes.

(**A**) Genes within conserved syntenic blocks have significantly lower MIS and MFS (Wilcoxon $p < 2.2e-16$). Syntenic genes or "syntenic block" is defined as at least three genes in the same order on the same chromosome as their orthologs in another species within a 25-kb search window. (**B** and **C**) Scatter plots show the insertion site enrichment along two syntenic breakpoints (Ch13:2,110,000 -2,135,000, Chr10: 642000-666000). Each gap in synteny (white area) is enriched for *piggyBac* insertions while flanked by essential regions (green shading); black boxes represent the location of CDS. (**D** and **E**) Circos plots indicate the syntenic blocks of *P. falciparum* in relation to other *Plasmodium spp.* (*P. berghei, P. chabaudi, P. knowlesi, P. vivax*).

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Fig. 5. Distinct biological process and evolutionarily conservation segregate the tendency of dispensable and essential genes.

(**A**) The genes with lowest FPKM expression value (first quantile) among different stages were enriched for dispensable genes (Wilcoxon P < 2.2e-16 compared with other quantiles)(*26*). The expression level cut off is set at 20 FPKM. (**B**) Non-mutable essential genes had significantly higher expression value for blood-stage development. (**C**) The group of trophozoite-stage genes had the highest proportion of essential genes (red) whereas gametocyte genes had the highest proportion of dispensable genes (blue) (Wilcoxon p < 1e-12). (**D** to **F**) Characteristics of essential genes significantly different from dispensable genes include: (D) 1:1 ortholog conserved among *Plasmodium spp*; (E) absence of paralogs; and (F) reduced rate of non-synonymous to synonymous SNPs. Bars indicate the group median ('****' indicates Wilcoxon p < 2.2e-16). (**G** and **H**) Essential genes reported in (G) *Toxoplasma* and (H) *P. berghei* showed significantly lower MIS in this mutagenesis screen of *P. falciparum* ('****' indicates Wilcoxon p < 2.2e-16). (**I**). Plot of Receiver Operating Characteristics (ROC) indicate the level of retention of essential genes across species. The MIS of *P. falciparum* more strongly correlates with the essentiality phenotype of *P. berghei* than *Toxoplasma*.

Fig. 6. Differentiating dispensable and essential genes and discovering high-priority druggable targets and pathways.

(A) Functional annotations of biological processes are represented by the p-value and the X-axis shows the fraction of the genes with MIS > 0.5. Each GO term is assigned a p-value on the Y-axis to represent the tendency to be essential or dispensable. Essentiality is indicated on a spectrum of red (essential) to blue (dispensable) and circle sizes indicate the GO term enrichment. (**B** and **C**) Boxplot of (B) molecular processes and (C) cellular components shows the MIS distribution generated by 1000x sampling of the number of genes in the query GO-term category. Left (red) and right (blue) triangles indicate GO terms with significantly lower

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or higher MIS (p-value < 0.05 compared to background), respectively; the heatmap represents the essentiality defined as the fraction of genes per GO term with MIS > 0.5.

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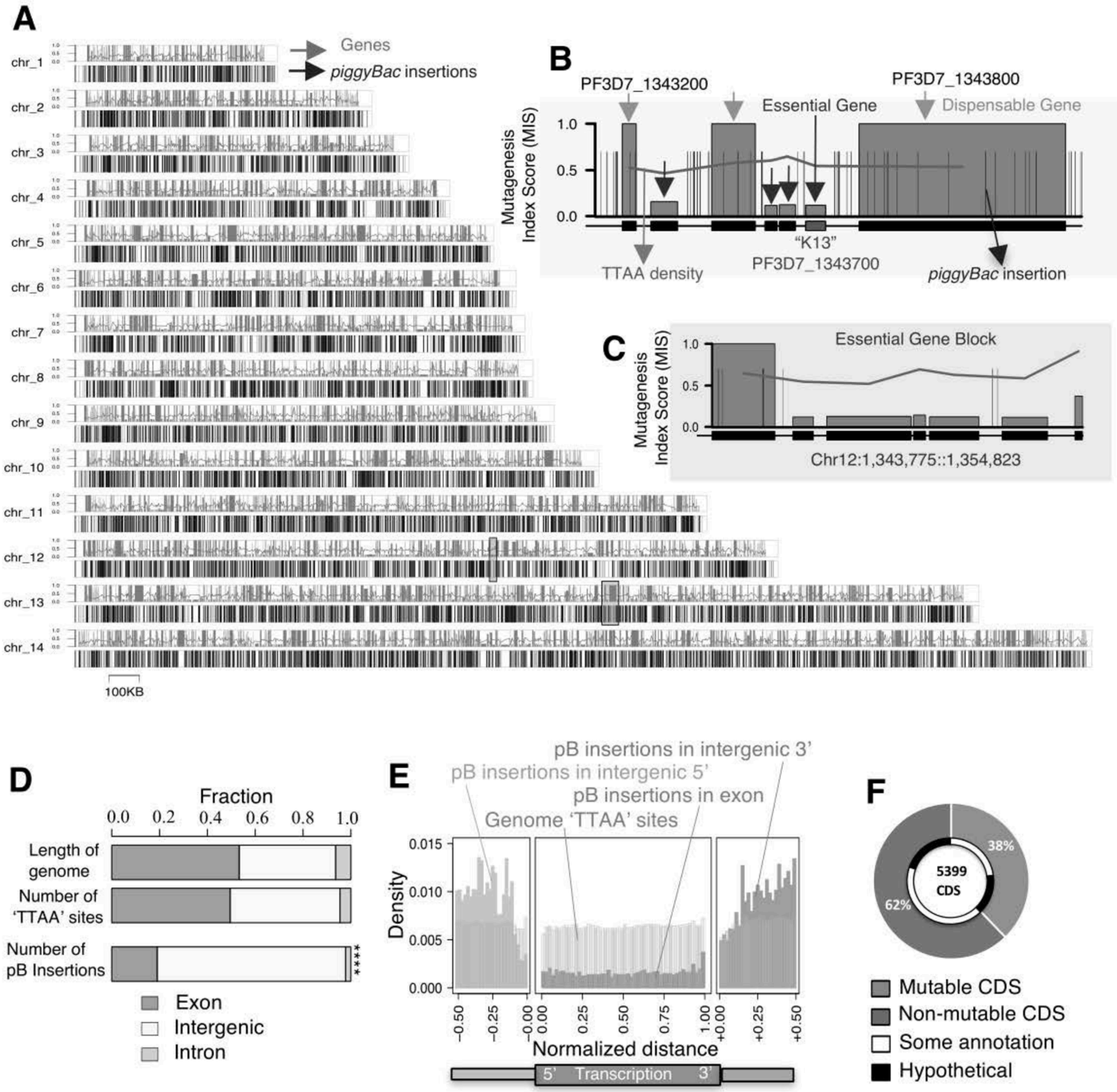

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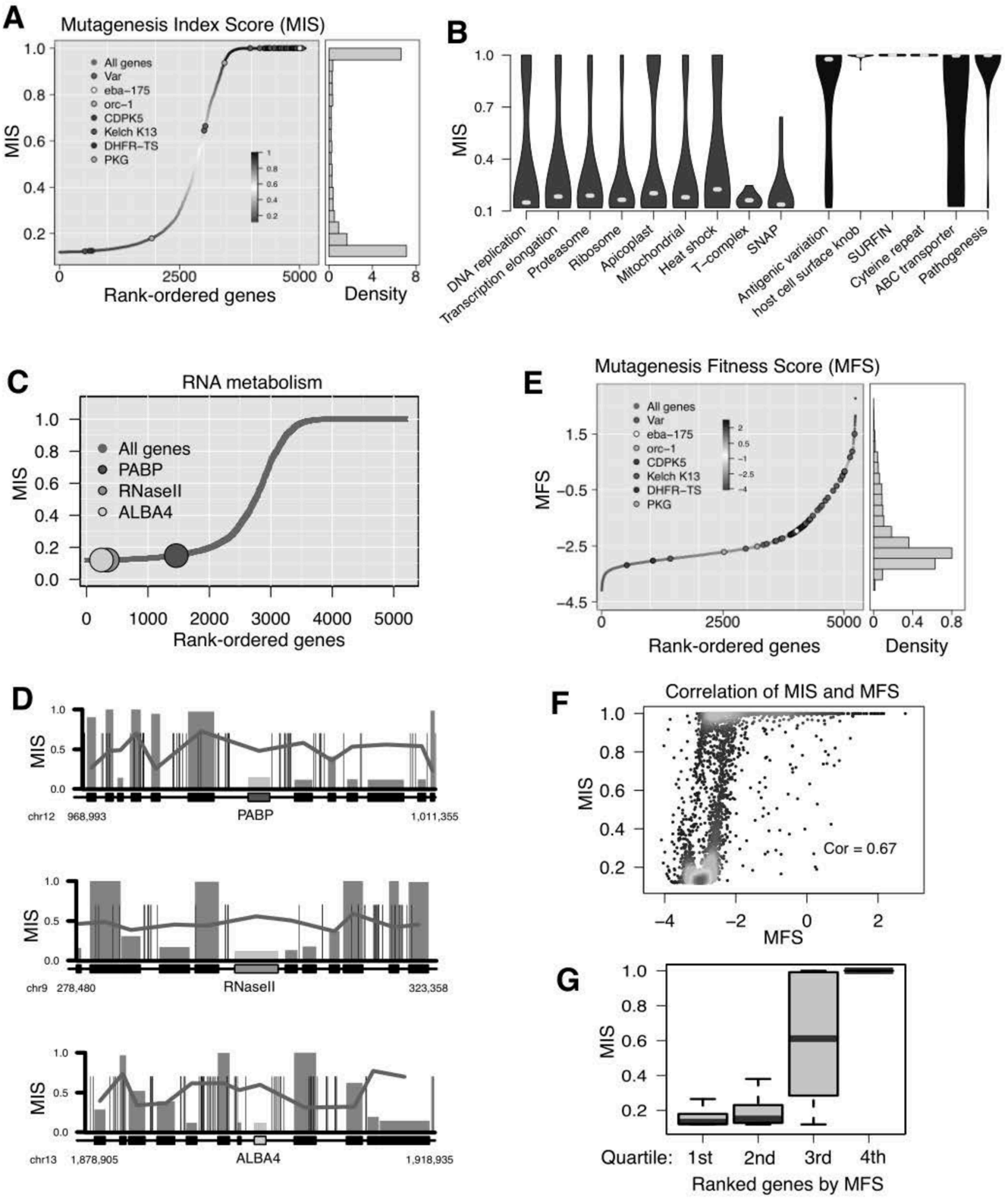

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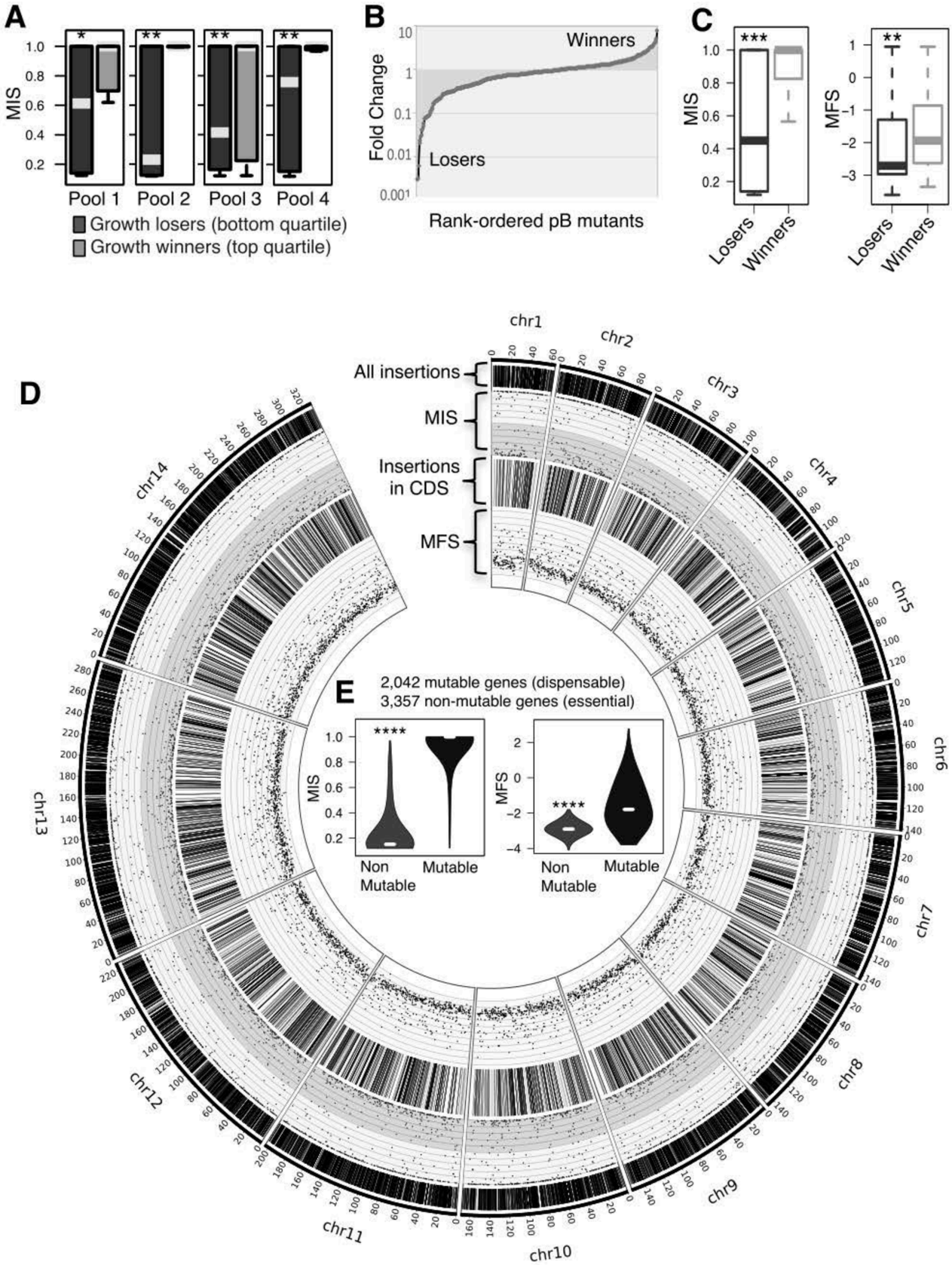

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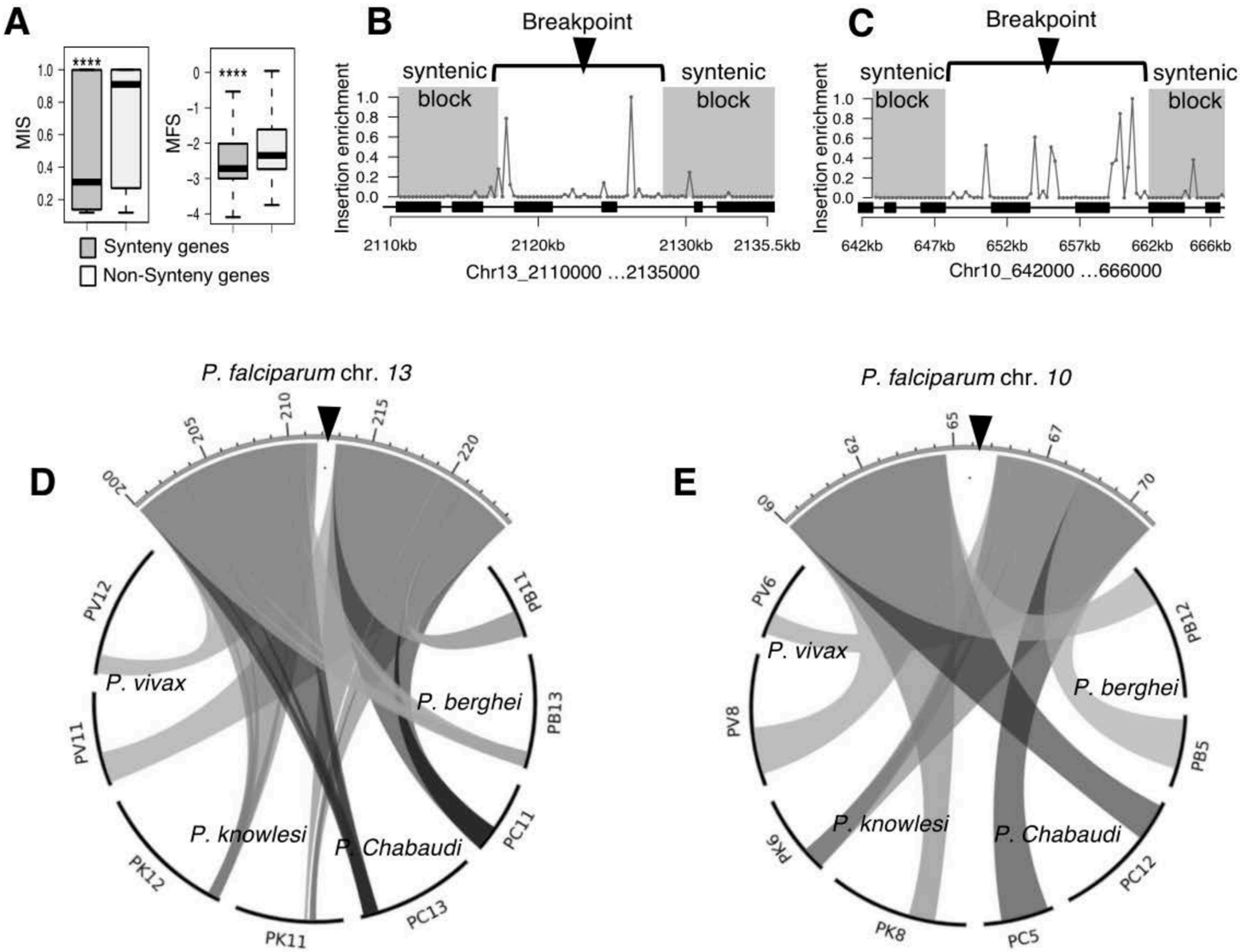

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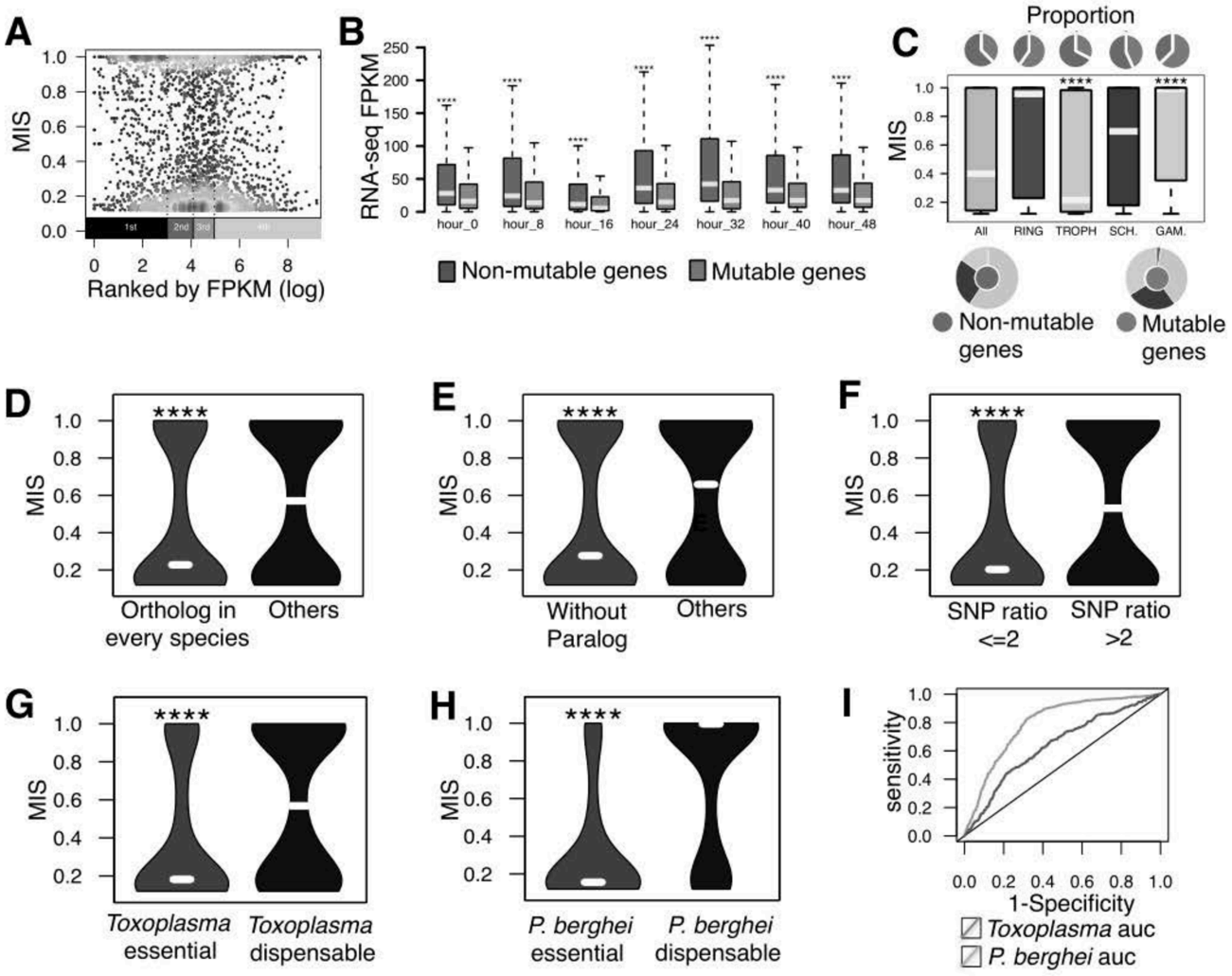

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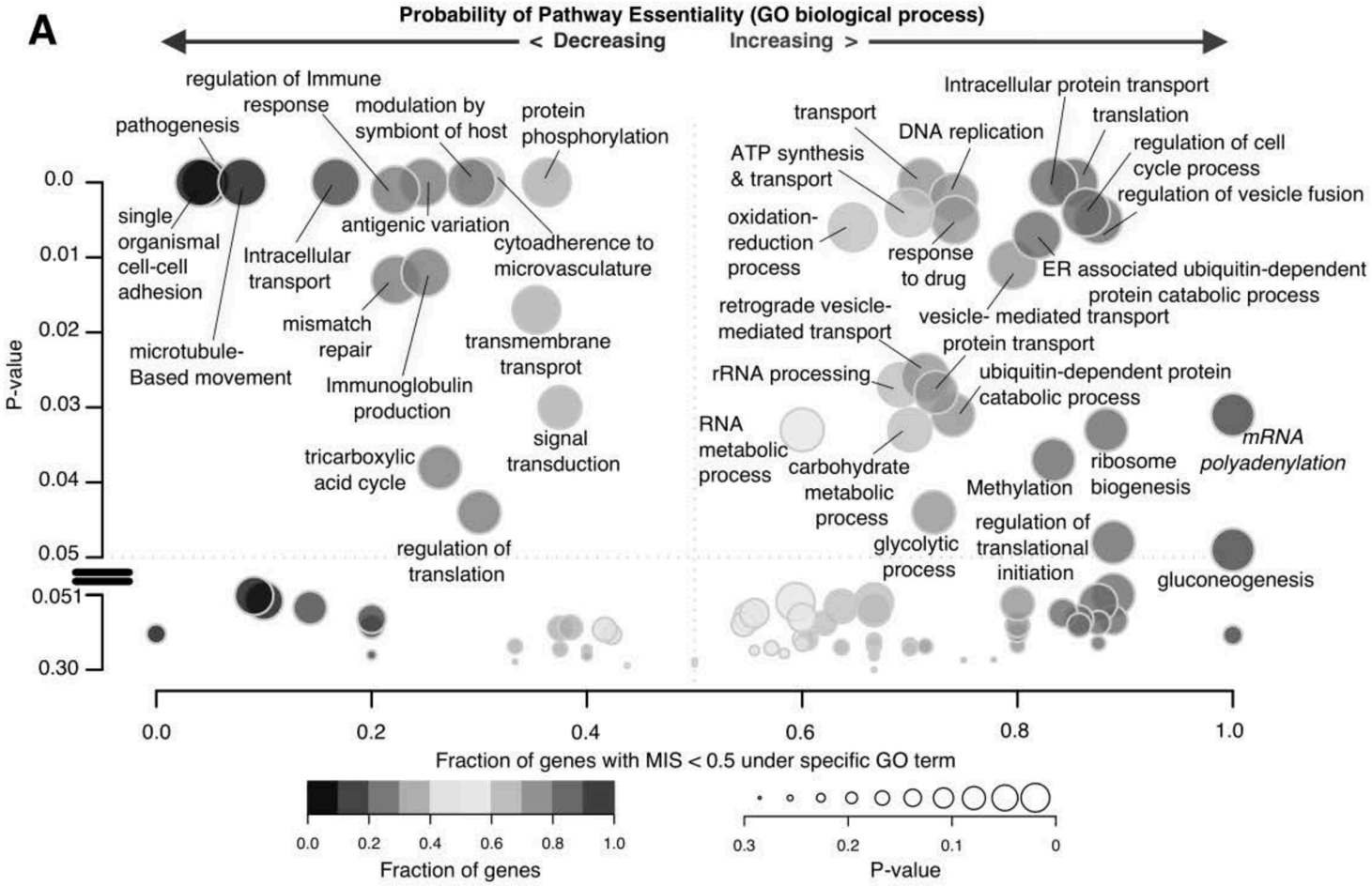

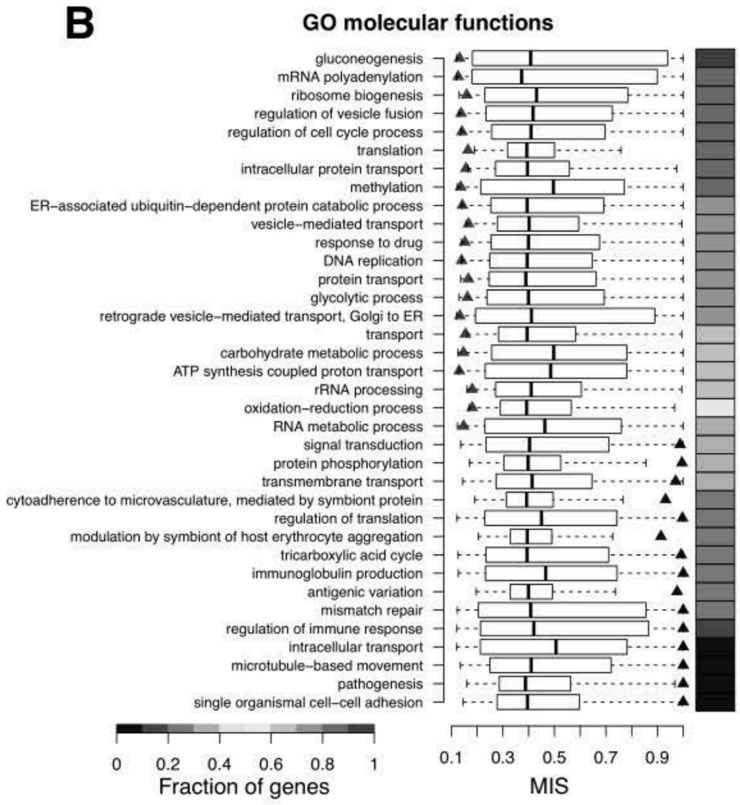

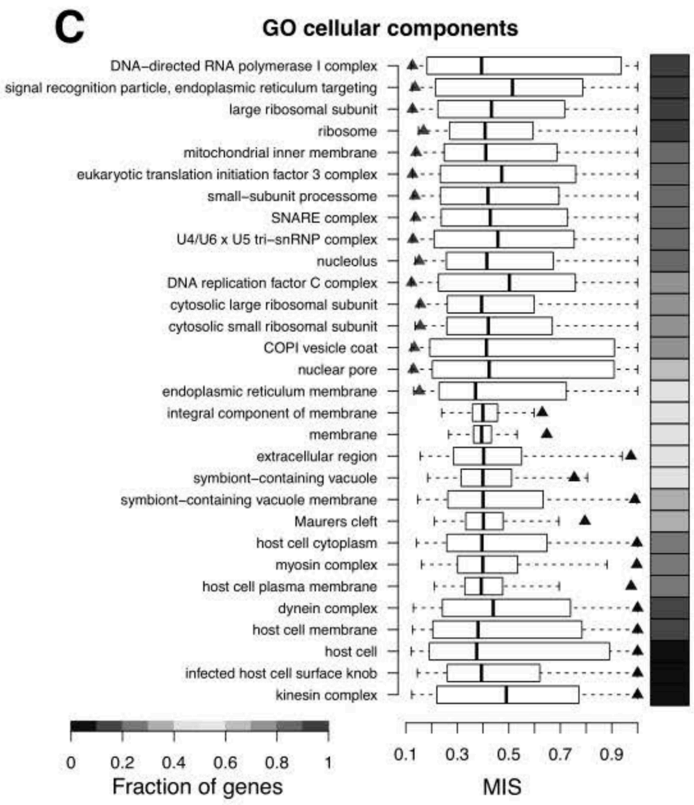

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Wed, 25 Apr 2018 17:53:17 +0000
To: Reid, Michael;Goosby, Eric
Cc: Eisinger, Robert (NIH/NIAID) [E];Doepel, Laurie (NIH/NIAID) [E]
Subject: Lancet Commission final pre-submission draft Review
Attachments: Lancet TB Report Comments 4.23.18.docx, Spectrum of TB Research and Development NIAID 12.19.17.pdf

Eric and Mike:

Thank you for the opportunity to review the entire draft Report. Your efforts to compile the feedback from all of the Commissioners into this draft should be applauded.

The first attachment labeled "Lancet TB Report Comments 4.23.18" lists significant comments and suggested edits in each section. I wanted to bring your attention a few of the most important of these, highlighted below:

- Executive Summary - Vision Statement – I would propose the following: "With bold leadership, accelerated research and development, implementation of evidence-based strategies, and a shared responsibility, we can end TB within a generation."
- Executive Summary - Priority 1 should be revised to include as the next to the last sentence "Special attention should be focused on TB and HIV coinfection."
- Executive Summary - Table 1 should be used rather than Figure 1.
- Section 2 – We propose an additional priority for this Section should be added -- "Invest in critical research on TB and HIV coinfection."
- Section 2 – Figure 2.0.1 should be used and not Figure 2.2.1. Two modifications should be considered to Figure 2.0.1 including: 1) the "Industry and Academic Partnerships" bar on the schematic could be larger reflecting its importance in the product development process; and 2) this bar on the schematic could be narrower in the "Basic Science/Biomarker" region and gradually get wider. The attached schematic which was provided previously illustrates the latter modification.

Additional comments and suggested edits can be found in each section of the Report in the tracked changes versions at the links below. As noted, in a few instances, figures/tables cited in the narrative are missing, or figures/tables that appear in the report are not cited in the narrative.

-

Executive Summary link https://file.ac/4vvxNEHLGyY/
Section 0 link https://file.ac/e4dGSd7FYVE/
Section 1 link https://file.ac/lpG4ovDsWYw/
Section 2 link https://file.ac/xQCyttQzkjE/
Section 3 link https://file.ac/D4R94Fn5hjk/
Section 4 link https://file.ac/Ue7pZHn3PoY/
Panels link https://file.ac/eJRGAfikEic/

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I suggest that the non-Commissioners who assisted in developing the Report should be clearly listed as "Contributors" immediately below the list of Commissioners who are the formal "authors". However, whatever you decide is fine with me. -

If we can provide any additional information or assistance, please let us know.
Best,
Tony

Anthony S. Fauci, MD
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National Institute of Allergy and Infectious Diseases
Building 31, Room 7A-03
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National Institutes of Health
Bethesda, MD 20892-2520
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E-mail: (b)(6)

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---

rom: Reid, Michael (b)(6)
Sent: Friday, April 20, 2018 3:14 AM
To: Goosby, Eric (b)(6)
Cc: Dean Jamison (b)(6) Soumya Swaminathan (b)(6)
Subject: Re: Lancet Commission final pre-submission draft Review

Dear Commissioners,

Thank you to all those of you that have contributed to revising the report and responding to the external reviewer comments. In particular, thanks to the teams from NIH and FIND for their help pulling pieces together!

Please find attached a zip file containing the current draft [minus a few pending analyses]. The full report – in 6 sections – can also be downloaded:

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(b)(4)

Best regards,

Mike

Lancet Commission on TB, Secretariat

From: "Reid, Michael" (b)(6)
Date: Sunday, April 15, 2018 at 06:22
To: Eric Goosby (b)(6)
Subject: Re: Lancet Commission Conf calls - week of April 16-20

Dear Commissioners,

Apologies for the delay in failing to circle back on this email from David sooner. Unfortunately, we were unable to find any date/time that worked for more than 2 or 3 people. With this in mind, Eric and I have decided to forgo a conference call for all Commissioners.

We are aiming to formally submit a revised draft of the Commission, as well as responses to all the external reviewers' comments, to The Lancet by **April 27**. In advance of that date, I intend to share a pre-submission draft of the full report to all Commissioners this coming Wed/Thurs [**April 18/19**]. I

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kindly ask that you all take time to review and make edits/comments – sending back any comments/edits no later than April 23.

I can personally be available for one-on-one calls on April 23 and 24 for anyone that wants to give feedback over the 'phone. Please email me individually if you would like to connect on either day.

Thanks for all of your input, particularly those of you that have been re-writing or reviewing subsections of the report over the last 2 weeks.

Kind regards,

Mike Reid
(on behalf of the Commission Secretariat)

From: David McKey (b)(6)
Date: Wednesday, April 4, 2018 at 09:52
To: David McKey (b)(6)
Cc: "Reid, Michael" (b)(6)
Subject: Lancet Commission Conf calls - week of April 16-20

Dear Commissioners,
As a follow-up to our recent meeting in New Delhi, it was agreed that we would arrange for two conference calls the week of April 16-20 to give an update on the progress and discuss any last minute details before the report is submitted the end of April.

Please find the link for a doodle poll indicating possible times for a call. Please select the two dates and times that work the best for you.
(b)(6)

If you plan on submitting expenses for reimbursement, please send as soon as possible. As an FYI, I will be out on holiday 10-22 April. Expenses not received before 6 April will be submitted upon my return to the office.

And, attached is the picture from our meeting.

Thank you,
David

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David McKey
Office of the UN Secretary-General's Special Envoy on Tuberculosis
Global Health Delivery & Diplomacy
Institute for Global Health Sciences | UCSF
550 16th Street | 3rd Floor | Box 1224
San Francisco, California 94158

(b)(6)
(b)(6)

UCSF Institute for Global Health Sciences

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Issues/Modifications for Lancet Commission Report (version 4.19.18)

Executive Summary (Recommendations/Priorities):

Lines 16-21 Vision Statement modification proposed: "With bold leadership, accelerated research and development, implementation of evidence-based strategies, and a shared responsibility, we can end TB within a generation."

Line 28 Delete comparison of priority on TB vs. HIV and Malaria.

Lines 49-50 Delete sentence citing the inadequate pace of TB R&D helped fuel DR-TB.

Line 110 Cites 3 million lives saved in Kenya; however, the narrative on Fig. 1C shows 4M and the graphic indicates 3M.

Lines 192-193 Priority 1 should be modified to include "Special attention should be focused on TB and HIV coinfection."

Figure 1 or Table 1 – Table 1 should be used rather than Figure 1. In either case, one needs to confirm that number of deaths averted in Kenya is 3M not 4M as cited above for Line 110.

Section 0 (Report Roadmap/Overview):

Figures 0.5.1 and 0.5.2 are not mentioned in the narrative but are included in this Section.

Line 126 Please clarify what the term "pro-poor" means.

Line 136 Please clarify what the phrase "protecting the TB risk pool" means.

Line 156-182 Confirm/add/update sources, dates, and data as needed for TB mortality and incidence. Some data through 2016 are from 2017 Global TB Report, other text references 2000-2015 data from Table 1.

Line 179 Table 0.4 is not found in this Section.

Line 247 A number needs to be assigned to Figure 0.x

Line 252 Needs to be corrected to 45% so that is matches the data in Fig. 0.4.

Line 258 Need to define NTP (National Tuberculosis [TB] Programme).

Line 268 Table 0.x is not included in this Section.

Section 1 (Strategies to reduce TB mortality):

Figures 1.3.1 There are two figures with this number.

Figures 1.5.1 There are two figures with this number.

Tables 1.3.2 There are two Tables with this number.

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Consider eliminating the Recommendations at the end of each subsection and fold any essential information they contain into the overall Recommendations at the beginning of Section 1. Subsection Recommendations lengthen Section 1 considerably and do not appear in other Sections.

Lines 62-77 Several priorities discussed in Section 1 are not included in the summary of policy priorities. Include them in first bullet or create a separate bullet to address. These priorities include establishing patient-centered care, closing the gaps in the TB care continuum, and instituting quality management.

Line 261-262 Please clarify "through peer exchange through international platforms for knowledge management."

Line 321 Please clarify/define the phrase "high-risk pools".

Line 357 There are two Figures labeled 1.3.1.

Lines 384-386 Please confirm this statement.

Line 553 Where is Figure 1?

Line 625 Where is Table 1.3.3?

Line 695 The title of the sub-section is not aligned with the focus of the narrative that follows.

Line 729 There are two Figures labeled 1.5.1, but this sentence matches with the first Figure, so it should be numbered 1.5.0.

Page 58-61 What narrative does this match with?

Section 2 (Research and Development):

Figure 2.2.3 Where does this match with the narrative?

Lines 25-35 In addition to the priorities provided in this section, an additional priority is "Invest in critical research on TB and HIV coinfection."

Line 49 Figure 2.0.1 should be included in the report. A slight modification to this Figure is proposed to accurately show that the "Industry and Academic Partnerships" bar on this schematic is larger; however, this component of the pipeline should begin gradually during the Basic Science/Biomarker Stage of the pipeline and then get larger as one moves down the pipeline. This gradual increase was reflected in the Schematic previously provided to Mike Reid and attached to the transmittal email.

Figure 2.2.1 should not be included in the report.

Section 3 (Macroeconomics):

Line 35 What is supposed to be included in this bullet?

Line 94 and 141 Please clarify/define the term "firms".

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Line 227 Delete sentence that compares funding for TB vs. HIV and malaria.

Line 263 Please clarify/define the term "pro-poor".

Section 4 (Enabling Strategies and Accountability):

Line 50 Please clarify/define the "four domains".

Line 128 Please clarify/define the term "built".

Lines 175-177 This sentence is awkward and needs to be rewritten.

Line 249 refers to Table 4.9.2, but it is not located in this section.

Panels (Background on Specific Topics):

Panel 2, #1. Please use a different term than "infantilize".

Panel 3, Replace "undiagnosed" for the term "missed".

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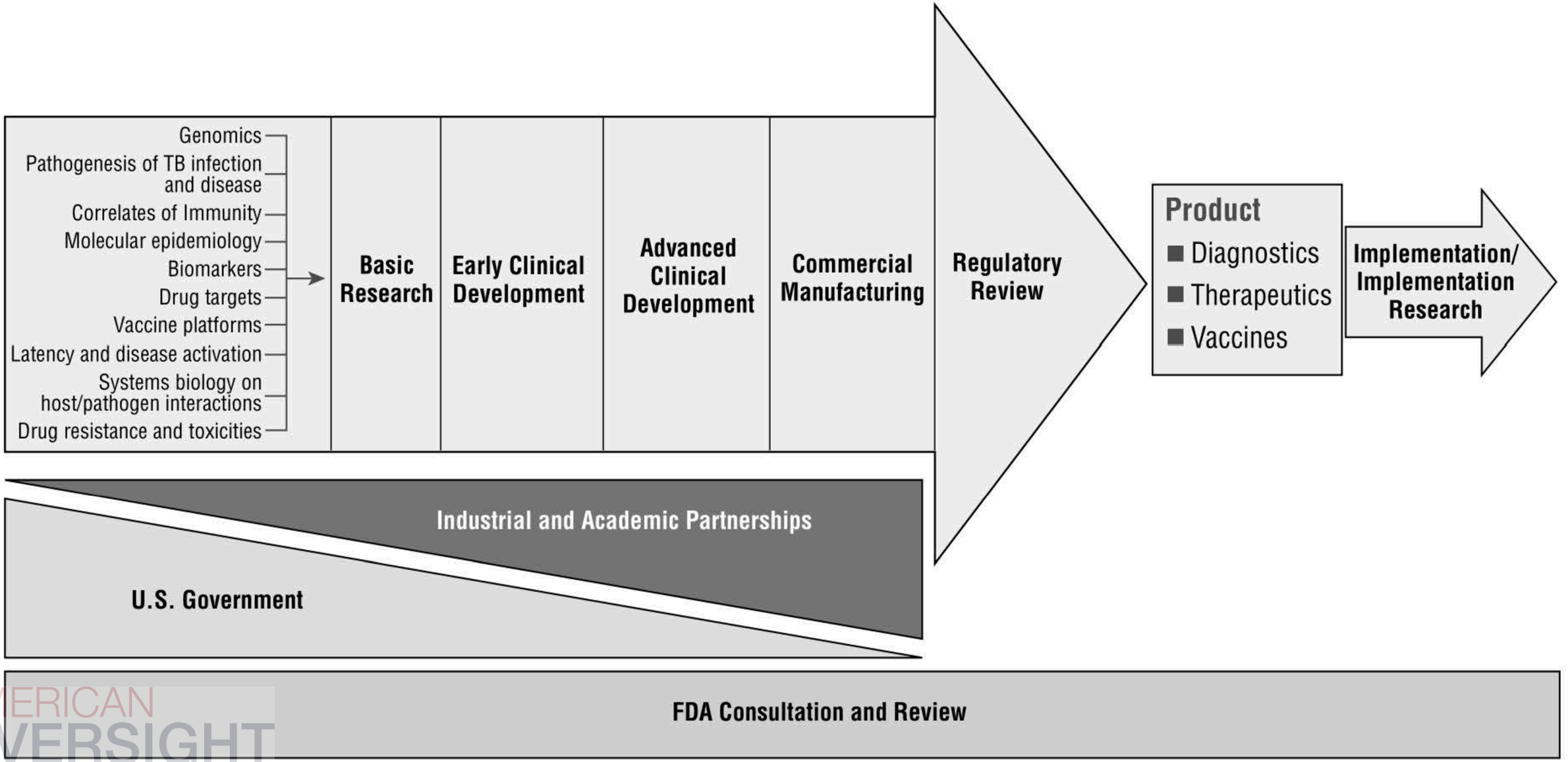

NIH/NIAID, 2017

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Wed, 25 Apr 2018 12:11:46 +0000
To: Folkers, Greg (NIH/NIAID) [E]
Cc: Conrad, Patricia (NIH/NIAID) [E]
Subject: RE: ASF -- 4th talk in Amsterdam FW: Invitation to participate in an IAS/AVAC journalist training at AIDS 2018

OK to assign to Bob.

From: Folkers, Greg (NIH/NIAID) [E]
Sent: Wednesday, April 25, 2018 7:53 AM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: ASF -- 4th talk in Amsterdam FW: Invitation to participate in an IAS/AVAC journalist training at AIDS 2018

RE note below
"I think if he could talk about the groundbreaking advances in px, tx and describe what is currently in the field (long-acting methods, vax trials, bNAbs, sustained virologic remission), that would be ideal."

Ok to assign this to Bob? It can be akin to the talk give to USCA or to Bopper Deyton's group

---

From: Rancourt, Anne (NIH/NIAID) [E]
Sent: Tuesday, April 24, 2018 7:15 PM
To: Folkers, Greg (NIH/NIAID) [E] (b)(6)
Cc: Conrad, Patricia (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6) Billet, Courtney (NIH/NIAID) [E] (b)(6) Stover, Kathy (NIH/NIAID) [E] (b)(6) NIAID OD AM (b)(6) Hoffman, Hillary (NIH/NIAID) [E] (b)(6)
Subject: Re: Invitation to participate in an IAS/AVAC journalist training at AIDS 2018

Yes — my understanding is that this is to provide some context for reporters to better prepare them to absorb the new science presented at the conference. I think if he could talk about the groundbreaking advances in px, tx and describe what is currently in the field (long-acting methods, vax trials, bNAbs, sustained virologic remission), that would be ideal. Most other speakers will focus on one specific area, so he's the best suited to explain how all these advances and pursuits can work together to bring an end to the pandemic.

On Apr 24, 2018, at 6:11 PM, Folkers, Greg (NIH/NIAID) [E] (b)(6) wrote:

On further reading, it is not really an aids history talk they are asking for / more of a "where we're at" talk
Do I have that right?
I have added to matrix

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"This session includes the personal reflections and observations of one of the most influential researchers and policy makers in HIV. Across nearly 35 years as director of the National Institute of Allergy and Infectious Disease (NIAID) at the US National Institutes of Health (NIH), Dr. Fauci has had one of the longest and most significant and influences on US and global research and policy development on HIV. In this session he offers a unique perspective on the greatest challenges and opportunities in HIV research, prevention, treatment and service delivery today."

From: Rancourt, Anne (NIH/NIAID) [E]
Sent: Tuesday, April 24, 2018 4:20 PM
To: Folkers, Greg (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Vance, Meaghan (NIH/NIAID) [C] (b)(6) Billet, Courtney (NIH/NIAID) [E] (b)(6) Stover, Kathy (NIH/NIAID) [E] (b)(6) NIAID OD AM (b)(6) Hoffman, Hillary (NIH/NIAID) [E] (b)(6)
Subject: Re: Invitation to participate in an IAS/AVAC journalist training at AIDS 2018

I think redundancy would be welcome for this group – it's intended to help prepare them for drinking from the firehose of information over the next few days.

From: "Folkers, Greg (NIH/NIAID) [E]" (b)(6)
Date: Tuesday, April 24, 2018 at 4:16 PM
To: Anne Rancourt (b)(6) "Conrad, Patricia (NIH/NIAID) [E]" (b)(6)
Cc: "Vance, Meaghan (NIH/NIAID) [C]" (b)(6) Courtney Billet (b)(6) Kathy Stover (b)(6) NIAID OD AM (b)(6) "Hoffman, Hillary (NIH/NIAID) [E]" (b)(6)
Subject: RE: Invitation to participate in an IAS/AVAC journalist training at AIDS 2018

07/22/18	Keynote at U=U: Celebrate, Activate and	Amsterdam
07/23/18	Janssen sponsored IAS - AIDS history talk	Amsterdam

Thanks
ASF has a similar, shorter (15 minute) talk the next day, so we can start thinking about beefing that one up a bit for the journalists
There will by necessity be some redundancy.

From: Rancourt, Anne (NIH/NIAID) [E]
Sent: Tuesday, April 24, 2018 4:07 PM
To: Folkers, Greg (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Vance, Meaghan (NIH/NIAID) [C] (b)(6) Billet, Courtney (NIH/NIAID) [E] (b)(6) Stover, Kathy (NIH/NIAID) [E] (b)(6) NIAID OD AM (b)(6) Hoffman, Hillary (NIH/NIAID) [E] (b)(6)
Subject: Re: Invitation to participate in an IAS/AVAC journalist training at AIDS 2018

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Correct. The audience is scholarship journalists who will likely be from southern Africa, India, and South America, as well as a few U.S. reporters with small budgets (think folks from The Body, or freelancers like Heather Boerner).

From: "Folkers, Greg (NIH/NIAID) [E]" (b)(6)
Date: Tuesday, April 24, 2018 at 3:59 PM
To: "Conrad, Patricia (NIH/NIAID) [E]" (b)(6) Anne Rancourt (b)(6)
Cc: "Vance, Meaghan (NIH/NIAID) [C]" (b)(6) Courtney Billet (b)(6) Kathy Stover (b)(6) NIAID OD AM (b)(6)
Subject: RE: Invitation to participate in an IAS/AVAC journalist training at AIDS 2018

This is another talk to be prepared, correct?

The proposed description for this session is: "This session includes the personal reflections and observations of one of the most influential researchers and policy makers in HIV. Across nearly 35 years as director of the National Institute of Allergy and Infectious Disease (NIAID) at the US National Institutes of Health (NIH), Dr. Fauci has had one of the longest and most significant and influences on US and global research and policy development on HIV. In this session he offers a unique perspective on the greatest challenges and opportunities in HIV research, prevention, treatment and service delivery today."

We would ask you to plan to present this session for approximately 25-30 minutes, and to leave 15-20 minutes or so for conversation and Q&A with the attending journalists

---

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Tuesday, April 24, 2018 3:57 PM
To: Mandy Sugrue (b)(6) Rancourt, Anne (NIH/NIAID) [E] (b)(6)
Cc: Aurigemma Mark (b)(6) Kay Marshall (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6) Billet, Courtney (NIH/NIAID) [E] (b)(6) Stover, Kathy (NIH/NIAID) [E] (b)(6)
Subject: RE: Invitation to participate in an IAS/AVAC journalist training at AIDS 2018

Hi Mandy:

Dr. Fauci would be happy to participate in your event on July 22 at 11:45 am. Dr. Fauci will likely use a powerpoint slide presentation so he would require a PC laptop at the podium so that he can closely see and

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advance his own slides. I will be the POC for his logistics so if you need anything else at this time, please let me know.

Thank you and I look forward to working with you and the IAS staff on this event.

Best,
-patty

Patricia L. Conrad
Special Assistant to the Director

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From: Mandy Sugrue (b)(6)
Sent: Tuesday, April 24, 2018 9:41 AM
To: Rancourt, Anne (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Aurigemma Mark (b)(6) Kay Marshall (b)(6)
Subject: Invitation to participate in an IAS/AVAC journalist training at AIDS 2018

Hi Anne and Patty,

I am writing to share an invitation for Dr. Fauci from IAS Executive Director Owen Ryan and AVAC Executive Director Mitchell Warren to be part of the faculty for an important new media capacity-building programme at AIDS 2018 called IAS/AVAC Journalist Fellows. Details on the programme and his requested participation are in the attached letter. We sincerely hope that he can add his voice to this important initiative, and look forward to hearing from you at your earliest possibility if he is available for this in Amsterdam. My colleagues Mark Aurigemma and Kay Marshall of AVAC are copied here and are coordinating the Journalist Fellowship programme at AIDS 2018. You can respond to them regarding this invitation, and with any questions. If you are able to be a part of this training, Mark and Kay will also follow up with more details on the event as the conference approaches.

Thank you for your consideration, and we hope to see you there.

Cheers,
Mandy

Mandy Sugrue

AMERICAN OVERSIGHT

Director, Communications
International AIDS Society

W: (b)(6) | M: (b)(6)

Avenue de France 23 | CH-1202 Geneva | Switzerland

iasociety.org @iasociety /iasociety

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From: Folkers, Greg (NIH/NIAID) [E]
Sent: Wed, 25 Apr 2018 11:52:31 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Conrad, Patricia (NIH/NIAID) [E]
Subject: ASF -- 4th talk in Amsterdam FW: Invitation to participate in an IAS/AVAC journalist training at AIDS 2018
Attachments: image001.png, image002.png, image003.png

RE note below
"I think if he could talk about the groundbreaking advances in px, tx and describe what is currently in the field (long-acting methods, vax trials, bNAbs, sustained virologic remission), that would be ideal."

Ok to assign this to Bob? It can be akin to the talk give to USCA or to Bopper Deyton's group

---

From: Rancourt, Anne (NIH/NIAID) [E]
Sent: Tuesday, April 24, 2018 7:15 PM
To: Folkers, Greg (NIH/NIAID) [E] (b)(6)
Cc: Conrad, Patricia (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6) Billet, Courtney (NIH/NIAID) [E] (b)(6) Stover, Kathy (NIH/NIAID) [E] (b)(6) NIAID OD AM (b)(6) Hoffman, Hillary (NIH/NIAID) [E] (b)(6)
Subject: Re: Invitation to participate in an IAS/AVAC journalist training at AIDS 2018

Yes — my understanding is that this is to provide some context for reporters to better prepare them to absorb the new science presented at the conference. I think if he could talk about the groundbreaking advances in px, tx and describe what is currently in the field (long-acting methods, vax trials, bNAbs, sustained virologic remission), that would be ideal. Most other speakers will focus on one specific area, so he's the best suited to explain how all these advances and pursuits can work together to bring an end to the pandemic.

On Apr 24, 2018, at 6:11 PM, Folkers, Greg (NIH/NIAID) [E] (b)(6) wrote:

On further reading, it is not really an aids history talk they are asking for / more of a "where we're at" talk
Do I have that right?
I have added to matrix

"This session includes the personal reflections and observations of one of the most influential researchers and policy makers in HIV. Across nearly 35 years as director of the National Institute of Allergy and Infectious Disease (NIAID) at the US National Institutes of Health (NIH), Dr. Fauci has had one of the longest and most significant and influences on US and global research and policy development on HIV. In this session he offers a unique perspective on the greatest challenges and opportunities in HIV research, prevention, treatment and service delivery today."

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From: Rancourt, Anne (NIH/NIAID) [E]
Sent: Tuesday, April 24, 2018 4:20 PM
To: Folkers, Greg (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Vance, Meaghan (NIH/NIAID) [C] (b)(6) Billet, Courtney (NIH/NIAID) [E] (b)(6) Stover, Kathy (NIH/NIAID) [E] (b)(6) NIAID OD AM (b)(6) Hoffman, Hillary (NIH/NIAID) [E] (b)(6)
Subject: Re: Invitation to participate in an IAS/AVAC journalist training at AIDS 2018

I think redundancy would be welcome for this group – it's intended to help prepare them for drinking from the firehose of information over the next few days.

From: "Folkers, Greg (NIH/NIAID) [E]" (b)(6)
Date: Tuesday, April 24, 2018 at 4:16 PM
To: Anne Rancourt (b)(6) "Conrad, Patricia (NIH/NIAID) [E]" (b)(6)
Cc: "Vance, Meaghan (NIH/NIAID) [C]" (b)(6) Courtney Billet (b)(6) Kathy Stover (b)(6) NIAID OD AM (b)(6) "Hoffman, Hillary (NIH/NIAID) [E]" (b)(6)
Subject: RE: Invitation to participate in an IAS/AVAC journalist training at AIDS 2018

07/22/18	Keynote at U=U: Celebrate, Activate and	Amsterdam
07/23/18	Janssen sponsored IAS - AIDS history talk	Amsterdam

Thanks
ASF has a similar, shorter (15 minute) talk the next day, so we can start thinking about beefing that one up a bit for the journalists
There will by necessity be some redundancy.

From: Rancourt, Anne (NIH/NIAID) [E]
Sent: Tuesday, April 24, 2018 4:07 PM
To: Folkers, Greg (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Vance, Meaghan (NIH/NIAID) [C] (b)(6) Billet, Courtney (NIH/NIAID) [E] (b)(6) Stover, Kathy (NIH/NIAID) [E] (b)(6) NIAID OD AM (b)(6) Hoffman, Hillary (NIH/NIAID) [E] (b)(6)
Subject: Re: Invitation to participate in an IAS/AVAC journalist training at AIDS 2018

Correct. The audience is scholarship journalists who will likely be from southern Africa, India, and South America, as well as a few U.S. reporters with small budgets (think folks from The Body, or freelancers like Heather Boerner).

From: "Folkers, Greg (NIH/NIAID) [E]" (b)(6)
Date: Tuesday, April 24, 2018 at 3:59 PM

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To: "Conrad, Patricia (NIH/NIAID) [E]" (b)(6) Anne Rancourt (b)(6)
Cc: "Vance, Meaghan (NIH/NIAID) [C]" (b)(6) Courtney Billet (b)(6) Kathy Stover (b)(6) NIAID OD AM (b)(6)
Subject: RE: Invitation to participate in an IAS/AVAC journalist training at AIDS 2018

This is another talk to be prepared, correct?

The proposed description for this session is: "This session includes the personal reflections and observations of one of the most influential researchers and policy makers in HIV. Across nearly 35 years as director of the National Institute of Allergy and Infectious Disease (NIAID) at the US National Institutes of Health (NIH), Dr. Fauci has had one of the longest and most significant and influences on US and global research and policy development on HIV. In this session he offers a unique perspective on the greatest challenges and opportunities in HIV research, prevention, treatment and service delivery today."

We would ask you to plan to present this session for approximately 25-30 minutes, and to leave 15-20 minutes or so for conversation and Q&A with the attending journalists

---

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Tuesday, April 24, 2018 3:57 PM
To: Mandy Sugrue (b)(6) Rancourt, Anne (NIH/NIAID) [E] (b)(6)
Cc: Aurigemma Mark (b)(6) Kay Marshall (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6) Billet, Courtney (NIH/NIAID) [E] (b)(6) Stover, Kathy (NIH/NIAID) [E] (b)(6)
Subject: RE: Invitation to participate in an IAS/AVAC journalist training at AIDS 2018

Hi Mandy:

Dr. Fauci would be happy to participate in your event on July 22 at 11:45 am. Dr. Fauci will likely use a powerpoint slide presentation so he would require a PC laptop at the podium so that he can closely see and advance his own slides. I will be the POC for his logistics so if you need anything else at this time, please let me know.

Thank you and I look forward to working with you and the IAS staff on this event.

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Best,
-patty

Patricia L. Conrad
Special Assistant to the Director

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From: Mandy Sugrue (b)(6)
Sent: Tuesday, April 24, 2018 9:41 AM
To: Rancourt, Anne (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Aurigemma Mark (b)(6) Kay Marshall (b)(6)
Subject: Invitation to participate in an IAS/AVAC journalist training at AIDS 2018

Hi Anne and Patty,

I am writing to share an invitation for Dr. Fauci from IAS Executive Director Owen Ryan and AVAC Executive Director Mitchell Warren to be part of the faculty for an important new media capacity-building programme at AIDS 2018 called IAS/AVAC Journalist Fellows. Details on the programme and his requested participation are in the attached letter. We sincerely hope that he can add his voice to this important initiative, and look forward to hearing from you at your earliest possibility if he is available for this in Amsterdam. My colleagues Mark Aurigemma and Kay Marshall of AVAC are copied here and are coordinating the Journalist Fellowship programme at AIDS 2018. You can respond to them regarding this invitation, and with any questions. If you are able to be a part of this training, Mark and Kay will also follow up with more details on the event as the conference approaches.

Thank you for your consideration, and we hope to see you there.

Cheers,
Mandy

Mandy Sugrue

Director, Communications
International AIDS Society

W: (b)(6) | M: (b)(6)

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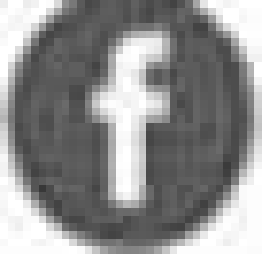

From: Holland, Steven (NIH/NIAID) [E]
Sent: Wed, 25 Apr 2018 02:44:13 +0000
To: Joyce, Maria (NIH/CC/OD) [E];Fauci, Anthony (NIH/NIAID) [E]
Cc: Lane, Cliff (NIH/NIAID) [E];Mascola, John (NIH/VRC) [E];Spisso, Suzanne (NIH/NIAID) [E];Eckes, Risa (NIH/NIAID) [E];Schacherer, Rachael (NIH/CC/OD) [E];Hiltbrand, Francine (NIH/CC/MRD) [E]
Subject: Re: NIAID FY 2018 Mid-Year Activity Report
Attachments: NIAID PI Branch Assignments-FY 18-corrected Holland.xlsx

I have made some edits to the spreadsheet.

Steve

--
Director, Division of Intramural Research
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bldg. 10/11N248 MSC 1960
Bethesda, MD 20892-1960
(b)(6) voice
301-480-4507 fax
(b)(6) email

Assistant lab: Eva Portillo
(b)(6) email
(b)(6) voice

Assistant to SD: Beth Schmidt
(b)(6)
(b)(6) voice

From: Maria Joyce (b)(6)
Date: Tuesday, April 24, 2018 at 10:01 AM
To: Anthony Fauci (b)(6)
Cc: (b)(6) (b)(6) "Lane, Cliff (NIH/NIAID) [E]" (b)(6) "Mascola, John (NIH/VRC) [E]" (b)(6) Suzanne Spisso (b)(6) Risa Eckes (b)(6) "Schacherer, Rachael (NIH/CC/OD) [E]" (b)(6) "Hiltbrand, Francine (NIH/CC/MRD) [E]" (b)(6)
Subject: NIAID FY 2018 Mid-Year Activity Report

Dear Dr. Fauci,

At this time, I would like to highlight the CC's patient activity and resource utilization through mid-year FY 2018.

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Attached you will find hospital patient activity data for FY 2018 mid-year, for the entire Clinical Center as well as your specific Institute. These data are included to provide insight on overall trends in patient activity, Institute-specific inpatient days and outpatient visits, average length of stay, and other key performance metrics. These data are snapshots of your census and activity dashboards found in the Executive Information System (EIS).

Additionally, to ensure that EIS accurately captures and reports IC activity by branch, please review the attached list of PI/Branch assignments and send any corrections to the EIS team.

Mid-Year Patient Activity Summary

In short, the CC has experienced a slight increase in inpatient days, average daily census, and outpatient visits compared to this time last year. ICs driving these increases are primarily NCI, NIMH and NIDDK in the inpatient setting; and NCI, NIAID, NICHD and NIAAA in the outpatient setting.

Fiscal Year	Mid-Year 2017	Mid-Year 2018	%Δ from FY2017
Inpatient Days	19,588	20,601	5%
Average Daily Census	107.6	113.2	5%
Average Length of Stay (ALOS)	8.6	8.4	-3%
Outpatient Visits	44,899	46,455	3%

If you would like to explore more detailed information for your Institute, we encourage you to contact the EIS team or, if you're an EIS user, visit http://eis.cc.nih.gov.

In conclusion, I hope this information is useful to you for managing your Institute's operations.

Thank you,
Maria

Maria D. Joyce
Chief Financial Officer
NIH Clinical Center
National Institutes of Health
Phone: (b)(6)
Fax: (301) 402-0244
(b)(6)

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Page 1583 of 2068

Withheld pursuant to exemption

(b)(5)

of the Freedom of Information and Privacy Act

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Page 1584 of 2068

Withheld pursuant to exemption

(b)(5)

of the Freedom of Information and Privacy Act

AMERICAN OVERSIGHT

Withheld pursuant to exemption

(b)(5)

of the Freedom of Information and Privacy Act

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Tue, 24 Apr 2018 19:51:51 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: IAS/AVAC Journalist Training Session (11:45 am - 12:30 pm)
Attachments: FW: Fauci: AIDS 2018 Journalist Fellowship Training Confirmation, FW: Invitation to participate in an IAS/AVAC journalist training at AIDS 2018, image679001.png

D203 of the conference venue, the Amsterdam RAI. Room D203 is located in the Elicium Centre section of the RAI complex. Use Door D to enter.
Contact Mark Aurigemma
(b)(6)
(b)(6)

The proposed description for this session is: "This session includes the personal reflections and observations of one of the most influential researchers and policy makers in HIV. Across nearly 35 years as director of the National Institute of Allergy and Infectious Disease (NIAID) at the US National Institutes of Health (NIH), Dr. Fauci has had one of the longest and most significant and influences on US and global research and policy development on HIV. In this session he offers a unique perspective on the greatest challenges and opportunities in HIV research, prevention, treatment and service delivery today."

We would ask you to plan to present this session for approximately 25-30 minutes, and to leave 15-20 minutes or so for conversation and Q&A with the attending journalists.

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Mon, 30 Apr 2018 18:03:29 +0000
To: Conrad, Patricia (NIH/NIAID) [E]
Subject: FW: Fauci: AIDS 2018 Journalist Fellowship Training Confirmation

Patricia L. Conrad
Special Assistant to the Director

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From: Aurigemma Mark (b)(6)
Sent: Monday, April 30, 2018 12:15 PM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Rancourt, Anne (NIH/NIAID) [E] (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6) Billet, Courtney (NIH/NIAID) [E] (b)(6) Stover, Kathy (NIH/NIAID) [E] (b)(6) Mandy Sugrue (b)(6) Kay Marshall (b)(6)
Subject: Fauci: AIDS 2018 Journalist Fellowship Training Confirmation

Dear Dr. Fauci:

Thank you for agreeing to be part of the IAS/AVAC Journalist Fellow training programme at AIDS 2018. We are delighted to confirm your participation in the event.

I will be working with Mandy Sugrue of IAS and Kay Marshall of AVAC to coordinate the training. I am writing to give you some background about the programme, and some details about your session.

Your session

Title: Summing up the field
Date: Sunday 22 July
Time: 11.45 am – 12.30 pm. Please plan to leave at least 10-15 minutes of your session for Q&A with the journalists.
Location: The training will take place at conference venue, the Amsterdam RAI. Room details to follow.

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Session description:

"This session includes the personal reflections and observations of one of the most influential researchers and policy makers in HIV. Across nearly 35 years as director of the National Institute of Allergy and Infectious Disease (NIAID) at the U.S. National Institutes of Health (NIH), Dr. Fauci has had one of the longest and most significant influences on U.S. and global research and policy development on HIV. In this session he offers a unique perspective on the greatest challenges and opportunities in HIV research, prevention, treatment and service delivery today."

If you wish to use slides, you can email those to me in advance, or bring the slides on a flash drive to the training. We will have a PC for your slides onsite. Please keep in mind that each training session is relatively brief, and plan the number of slides you will use accordingly.

We will follow-up closer to the training date with more information on the training location and logistics.

Your audience

The twenty Journalist Fellows taking part in this first IAS/AVAC training programme were selected from among the pool of AIDS 2018 media scholarship recipients. They represent a cross-section of print, broadcast and online journalists from low- and middle-income countries.

All of the Journalist Fellows have a demonstrated commitment to reporting on HIV and related issues, but their levels of scientific knowledge will vary. Some are highly experienced in HIV science, while others have focused more on community-based responses to the epidemic. Also, while all of our Fellows are proficient in English, for a significant number English is a second or third language. It is always helpful to check in with the Fellows during your presentation to ensure that they are following along, and to see if they have any questions.

Kay Marshall or I will be in touch before the training with more information, including a full programme for the event. In the interim, if you have any questions or would like to discuss anything about the training, or if there is anything I can do to help you prepare, please let me know.

Thanks, again, for being part of this. We truly appreciate it.

Mark

Mark Aurigemma
(b)(6)
(b)(6)

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Fri, 1 Jun 2018 18:35:43 +0000
To: Conrad, Patricia (NIH/NIAID) [E]
Subject: FW: Invitation to participate in an IAS/AVAC journalist training at AIDS 2018
Attachments: Anthony Fauci - AIDS 2018 Journalist Training invite_MS.DOCX

Patricia L. Conrad
Public Health Analyst and
Special Assistant to the Director
National Institute of Allergy and Infectious Diseases
The National Institutes of Health
31 Center Drive, MSC 2520 - Room 7A03
Bethesda, Maryland 20892
(b)(6)
301-496-4409 fax

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From: Mandy Sugrue (b)(6)
Sent: Tuesday, April 24, 2018 9:41 AM
To: Rancourt, Anne (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: Aurigemma Mark (b)(6) Kay Marshall (b)(6)
Subject: Invitation to participate in an IAS/AVAC journalist training at AIDS 2018

Hi Anne and Patty,

I am writing to share an invitation for Dr. Fauci from IAS Executive Director Owen Ryan and AVAC Executive Director Mitchell Warren to be part of the faculty for an important new media capacity-building programme at AIDS 2018 called IAS/AVAC Journalist Fellows. Details on the programme and his requested participation are in the attached letter. We sincerely hope that he can add his voice to this important initiative, and look forward to hearing from you at your earliest possibility if he is available for this in Amsterdam. My colleagues Mark Aurigemma and Kay Marshall of AVAC are copied here and are coordinating the Journalist Fellowship programme at AIDS 2018. You can respond to them regarding this invitation, and with any questions. If you are able to be a part of this training, Mark and Kay will also follow up with more details on the event as the conference approaches.

Thank you for your consideration, and we hope to see you there.

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Cheers,
Mandy

Mandy Sugrue

Director, Communications
International AIDS Society

W: +41 22 710 08 43 | M: (b)(6)

Avenue de France 23 | CH-1202 Geneva | Switzerland

iasociety.org @iasociety /iasociety

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Tue, 24 Apr 2018 18:31:00 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: Assistant Secretary for Health, Brett Giroir VRC Tour
Attachments: FW: Brett Giroir, RE: May 3rd visit , FW: ASH Dr. Brett Giroir's visit to NIH this Thursday

Amy Blackburn (b)(6)
Alan Chan (b)(6) (this cell # will be the guaranteed way to reach me on the day of)
Ashley Parker (b)(6) (work cell)
Carol Jabir (b)(6) (work cell)

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Tue, 24 Apr 2018 18:27:05 +0000
To: Vance, Meaghan (NIH/NIAID) [C]
Subject: FW: Brett Giroir

Patricia L. Conrad
Special Assistant to the Director

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From: Burklow, John (NIH/OD) [E]
Sent: Tuesday, March 27, 2018 4:22 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Conrad, Patricia (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Subject: RE: Brett Giroir

Thanks, Dr. Fauci! Will do!

John

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Tuesday, March 27, 2018 1:58 PM
To: Burklow, John (NIH/OD) [E] (b)(6)
Cc: Conrad, Patricia (NIH/NIAID) [E] (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6)
Subject: RE: Brett Giroir

John:
Thanks for the note. I would be happy to meet with Dr. Giroir. I agree that we could show him the VRC or the SCSU. We can decide as we firm up the date. As you suggest, please work out a time with Patty.
Best,
Tony

From: Burklow, John (NIH/OD) [E]
Sent: Tuesday, March 27, 2018 1:56 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)

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Cc: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: Brett Giroir

Hi, Dr. Fauci—

Francis has invited the Assistant Secretary for Health, Dr. Brett Giroir, out to NIH for a visit. We haven't set a date yet. I'm writing to see if you'd like to meet with him and perhaps show him either the VRC or the Special Studies Unit. If so, we'll work out a time with Patty, etc.

Thanks,

John

John Burklow
Associate Director for Communications and Public Liaison
National Institutes of Health
1 Center Drive/Building 1
Room 344
9000 Rockville Pike
Bethesda, MD 20892
Phone: (b)(6)
Cell: (b)(6)
(b)(6)

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From: McManus, Ayanna (NIH/OD) [E]
Sent: Tue, 24 Apr 2018 20:55:58 +0000
To: Vance, Meaghan (NIH/NIAID) [C]
Cc: Conrad, Patricia (NIH/NIAID) [E]
Subject: RE: May 3rd visit

Many thanks, Meaghan! Yes, please hold 10AM - 12PM on the calendar until we sort through the details. The tour will be 45 minutes.

I'll ask about us coordinating the visit with the VRC and will let you know.

From: Vance, Meaghan (NIH/NIAID) [C]
Sent: Tuesday, April 24, 2018 2:36 PM
To: McManus, Ayanna (NIH/OD) [E] (b)(6)
Cc: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: RE: May 3rd visit

Also, will building 1 be coordinating the visit with VRC?

From: Vance, Meaghan (NIH/NIAID) [C]
Sent: Tuesday, April 24, 2018 2:31 PM
To: McManus, Ayanna (NIH/OD) [E] (b)(6)
Cc: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Subject: May 3rd visit

Hi Ayanna:

Dr. Fauci is available on May 3rd for the tour of the VRC with Brett Giroir. Just to confirm – we will hold 10-12pm and the VRC tour will be 45 minutes within that block of time.

Thanks,

Meaghan Vance
Office of the Director
(b)(6)
(b)(6)

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From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Wed, 2 May 2018 17:09:00 +0000
To: Vance, Meaghan (NIH/NIAID) [C]
Cc: Greg Folkers (b)(6)
Subject: FW: ASH Dr. Brett Giroir's visit to NIH this Thursday
Attachments: Draft Minute by minute agenda ADM Brett Giroir Visit 5_3_18 4_30_18_.docx

Please replace this as final agenda in the folder. ASF should arrive VRC around 11 am.

thx

Patricia L. Conrad
Special Assistant to the Director

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Amy

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Sent: Wednesday, May 02, 2018 10:56 AM
To: Blackburn, Amy (NIH/OD) [E] (b)(6)
Subject: RE: ASH Dr. Brett Giroir's visit to NIH this Thursday

AMERICAN OVERSIGHT

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Sent: Tuesday, May 01, 2018 4:21 PM
To: Hurlebaus, Lisa (NIH/OD) [E] (b)(6) Parker, Ashley (NIH/OD) [E] (b)(6)

AMERICAN OVERSIGHT

Cc: Burklow, John (NIH/OD) [E] (b)(6) Blackburn, Amy (NIH/OD) [E] (b)(6) Baker, Rebecca (NIH/OD) [E] (b)(6) Allen-Gifford, Patrice (NIH/OD) [E] (b)(6) Doswell, Greta (NIH/OD) [E] (b)(6) Gale, Jamie (NIH/OD) [E] (b)(6) McManus, Ayanna (NIH/OD) [E] (b)(6)
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Carrie D. Wolinetz, Ph.D.
Acting Chief of Staff and
Associate Director for Science Policy
Office of the Director
National Institutes of Health
(b)(6)

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AMERICAN OVERSIGHT

From: Wellman, Magdalene (NIH/OD) [C] on behalf of Goodenow, Maureen (NIH/OD) [E]
Sent: Tue, 24 Apr 2018 18:46:41 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: NIAID Budget Group;Dieffenbach, Carl (NIH/NIAID) [E];Palmer, Robert (NIH/NIAID) [E]
Subject: FY 2018 Allocation and FY 2019 President's Budget Levels
Attachments: ALLOCATION LETTER TEMPLATE_NIAID.DOCX

Please see attached the FY 2018 HIV/AIDS Allocation and the FY 2019 HIV/AIDS President's Budget memo which contains details of your ICO funding. For any questions, please contact the OAR Budget Office at (b)(6)

Best Regards,

Maureen M. Goodenow, Ph.D.
Director, Office of AIDS Research
Associate Director for AIDS Research
National Institutes of Health

AMERICAN OVERSIGHT

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20892

DATE: April 15, 2021

TO: Anthony S. Fauci, M.D.
Director, NIAID

FROM: Director, Office of AIDS Research

SUBJECT: FY 2018 HIV/AIDS Allocation and FY 2019 President's Budget Estimate

On behalf of the Office of AIDS Research (OAR), I would like to thank you and your Institute for your continued support and cooperation over the past year. With the passage of the FY 2018 Consolidated Appropriations Act and with the submission of the FY 2019 President's Budget request to Congress, the OAR is providing the NIAID its FY 2018 allocation and FY 2019 President's Budget for HIV/AIDS research.

The Trans-NIH Plan for HIV and HIV-Related Research (the plan) has the following overarching priorities:

1) **Reducing Incidence of HIV**
2) **Research Toward HIV Cure**
3) **Develop Next Generation Therapies**
4) **Address HIV-Associated Comorbidities, Coinfections, and Complications**
5) **Cross-Cutting Priorities:**
    - **Basic Research**
    - **Behavioral and Social Sciences Research**
    - **Health Disparities Research**
    - **Information Dissemination**
    - **Research Training**

This framework of priorities encompasses the OAR's vision to end the HIV/AIDS pandemic and improve health outcomes for people living with, or at risk for, HIV. The OAR is responsible for allocating funds to advance the Trans-NIH HIV/AIDS research activities to accomplish these goals.

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FY 2018 HIV/AIDS Budget at the Consolidated Appropriations Act Level:

The HIV/AIDS budget for the NIAID is $1,687,986,000. This allocation is in alignment with the overarching, high priority HIV/AIDS research areas.

The FY 2018 HIV/AIDS Funding table for the NIAID is attached for your information.

FY2019 HIV/AIDS Budget at the Revised President's Budget Request Level:

At the FY 2019 President's Budget request level, the HIV/AIDS allocation for the NIAID is $1,624,190,000.

The investment in HIV/AIDS research continues to produce critical scientific advances. We appreciate your dedication, continued support, and cooperation as we work together to advance the NIH HIV/AIDS research program and end the HIV/AIDS pandemic. If you have any questions, please feel free to contact me.

4/24/2018

X

Maureen M. Goodenow, Ph.D.
Director, Office of AIDS Research
Signed by: Maureen M. Goodenow -S

Attachments:
FY 2018 HIV/AIDS Allocation Table
FY 2018 HIV/AIDS Approved Initiatives
FY 2019 HIV/AIDS Approved Initiatives

NIAID FY 18 HIV AIDS Allocation Tabl

NIAID FY 18 Approved Initiatives

NIAID FY 19 Approved Initiatives

cc:
NIAID Budget Office
NIAID AIDS Coordinator

AMERICAN OVERSIGHT

From: Joyce, Maria (NIH/CC/OD) [E]
Sent: Tue, 24 Apr 2018 14:00:56 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Holland, Steven (NIH/NIAID) [E];Lane, Cliff (NIH/NIAID) [E];Mascola, John (NIH/VRC) [E];Spisso, Suzanne (NIH/NIAID) [E];Eckes, Risa (NIH/NIAID) [E];Schacherer, Rachael (NIH/CC/OD) [E];Hiltbrand, Francine (NIH/CC/MRD) [E]
Subject: NIAID FY 2018 Mid-Year Activity Report
Attachments: NIAID Mid-Year 2018 Report.pdf, NIAID PI Branch Assignments-FY 18.xlsx

Dear Dr. Fauci,

At this time, I would like to highlight the CC's patient activity and resource utilization through mid-year FY 2018.

Attached you will find hospital patient activity data for FY 2018 mid-year, for the entire Clinical Center as well as your specific Institute. These data are included to provide insight on overall trends in patient activity, Institute-specific inpatient days and outpatient visits, average length of stay, and other key performance metrics. These data are snapshots of your census and activity dashboards found in the Executive Information System (EIS).

Additionally, to ensure that EIS accurately captures and reports IC activity by branch, please review the attached list of PI/Branch assignments and send any corrections to the EIS team.

Mid-Year Patient Activity Summary

In short, the CC has experienced a slight increase in inpatient days, average daily census, and outpatient visits compared to this time last year. ICs driving these increases are primarily NCI, NIMH and NIDDK in the inpatient setting; and NCI, NIAID, NICHD and NIAAA in the outpatient setting.

Fiscal Year	Mid-Year 2017	Mid-Year 2018	%Δ from FY2017
Inpatient Days	19,588	20,601	5%
Average Daily Census	107.6	113.2	5%
Average Length of Stay (ALOS)	8.6	8.4	-3%
Outpatient Visits	44,899	46,455	3%

If you would like to explore more detailed information for your Institute, we encourage you to contact the EIS team or, if you're an EIS user, visit http://eis.cc.nih.gov.

In conclusion, I hope this information is useful to you for managing your Institute's operations.

Thank you,

AMERICAN OVERSIGHT

Maria

Maria D. Joyce
Chief Financial Officer
NIH Clinical Center
National Institutes of Health
Phone: (b)(6)
Fax: (301) 402-0244
(b)(6)

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Withheld pursuant to exemption

(b)(5)

of the Freedom of Information and Privacy Act

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Page 1612 of 2068

Withheld pursuant to exemption

(b)(5)

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Page 1613 of 2068

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Page 1614 of 2068

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Page 1616 of 2068

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Page 1619 of 2068

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AMERICAN OVERSIGHT

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Sent: Tue, 24 Apr 2018 12:54:47 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Folkers, Greg (NIH/NIAID) [E];Eisinger, Robert (NIH/NIAID) [E];Vance, Meaghan (NIH/NIAID) [C];Conrad, Patricia (NIH/NIAID) [E];Paules, Catharine (NIH/NIAID) [E];Doepel, Laurie (NIH/NIAID) [E]
Subject: FW: AMP slides
Attachments: Clinical MAb slides for IAVI mtg v3.pptx

This is Julie's presentation on VRC HIV bNAb program (including a big focus on AMP). Lots of good material.

From: Ledgerwood, Julie (NIH/NIAID) [E]
Sent: Monday, April 16, 2018 2:48 PM
To: Marston, Hilary (NIH/NIAID) [E] (b)(6) Sitar, Sandra (NIH/VRC) [C] (b)(6)
Subject: RE: AMP slides

I have attached my slides from Friday---feel free to use any.

From: Marston, Hilary (NIH/NIAID) [E]
Sent: Monday, April 16, 2018 1:17 PM
To: Ledgerwood, Julie (NIH/NIAID) [E] (b)(6)
Subject: AMP slides

Hey Julie – Would you mind sharing some of your AMP slides? I wanted to weddle-ize the map at least (with attribution of course). Will send you the weddle versions.

Hilary Marston, MD, MPH
Medical Officer and Policy Advisor for Global Health
NIAID Office of the Director
(b)(6)
(b)(6) (cell)

AMERICAN OVERSIGHT

National Institute of Allergy and Infectious Diseases
Dale and Betty Bumpers Vaccine Research Center

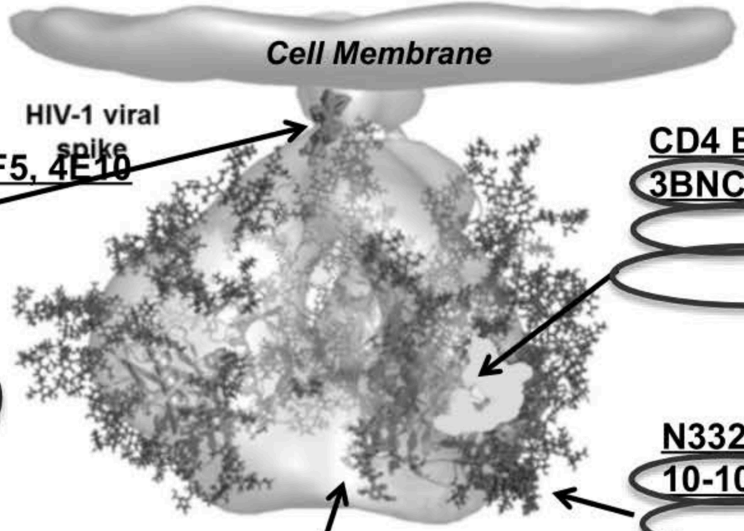
VRC HIV MAbs in Clinical Trials

Julie E. Ledgerwood, D.O.ChiefClinical Trials Program Vaccine Research CenterNational Institute of Allergy and Infectious DiseasesNational Institutes of Health

AMERICAN OVERSIGHT

HIV-1 Neutralizing mAb Products in Clinical Development

4 major sitesin 2018



gp41 MPER:2F5, 4E10

10e8v5R

Bi-specific (10e8/anti-CD4)

Tri-specific (CD4bs, V2, MPER)

eCD4-Ig (CD4, anti-CCR5)

CD4 Binding Site:VRC01, VRC01-LS, CH31
3BNC117, 12A12, CH103, VRC07-523LS, Z

N332 Glycan Supersite:PGT121, PGT128
10-1074

V1V2 Glycan:PG6, PG16, CH01-04
PGT141-45, PGDM1400
CAP256-VRC26

Phase 1

GMP manufacturing

AMERICAN OVERSIGHT

Cryo-EM of viral spike by Subramaniam group. Fit with atomic level structures from Kwong and Wilson groups

se 1/1b Trials Summary(VRC 601, VRC 602

Sep 24, 2015

Clinical & Experimental Immunology

Safety, Pharmacokinetics and Neutralization of the Broadly Neutralizing HIV-1 Human Monoclonal Antibody VRC01 in Healthy adults.Ledgerwood JE, Coates EE, et al., Clin Exp Immunol. 2015

Dec 23, 2015

Science Translational Medicine

Virologic effects of broadly neutralizing antibody VRC01 administration during chronic HIV-1 infection.Lynch RM, Boritz E, Coates EE, et al, Sci Transl Med. 2015 Dec 23;7(319):319ra206.

Nov 14, 2017

PLOS | MEDICINE

Safety, pharmacokinetics, and immunological activities of multiple intravenous or subcutaneous doses of an anti-HIV monoclonal antibody, VRC01, administered to HIV-uninfected adults: Results of a phase 1 randomized trial.Mayer et al. PLoS Med. 2017 Nov 14;14(11):e1002435.

- VRC01 safe Serum concentrations of >20 mcg/mL maintained for 8 weeks at 20 mg/kg doseNo anti-drug antibody VRC01 half life: 15 daysVRC01 reduced VL by >1 Log in 6/8 viremic subjectsVRC01 Advanced to Phase 2b efficacy testing in AMP

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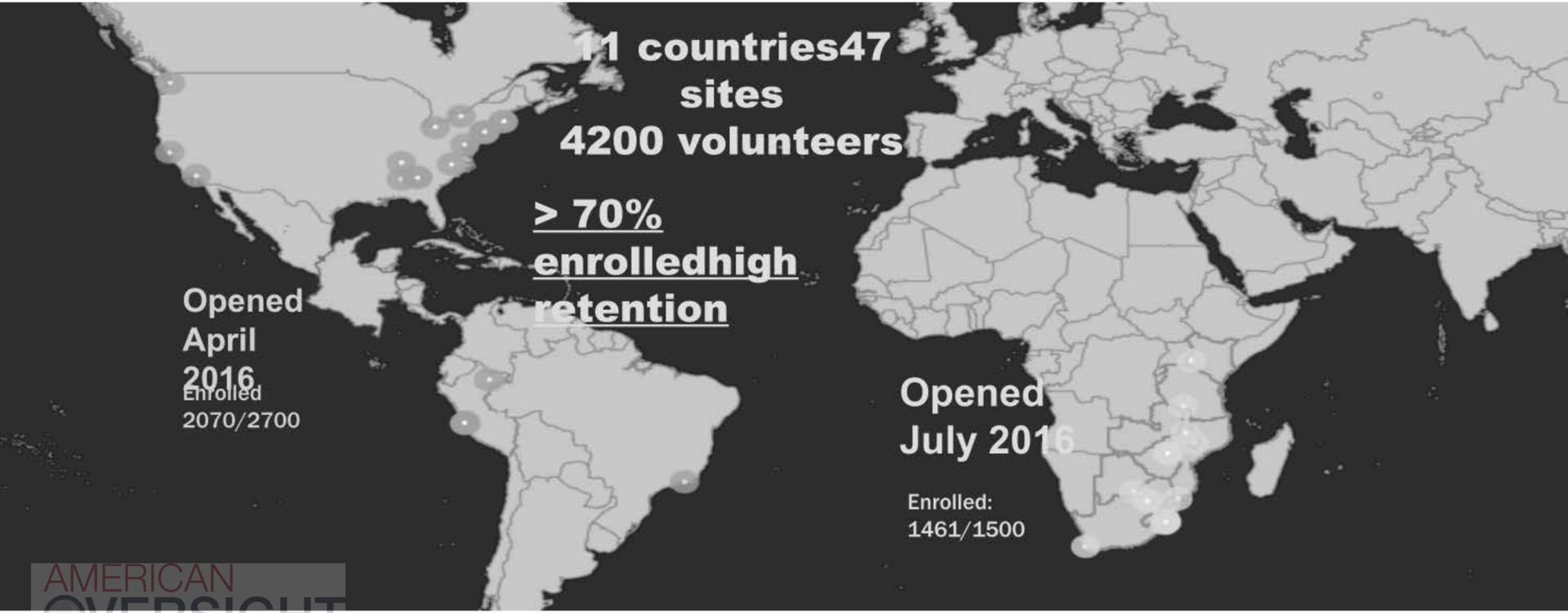
Antibody-Mediated Prevention (AMP)

VRC01 mAb phase 2b studies in high risk men and women



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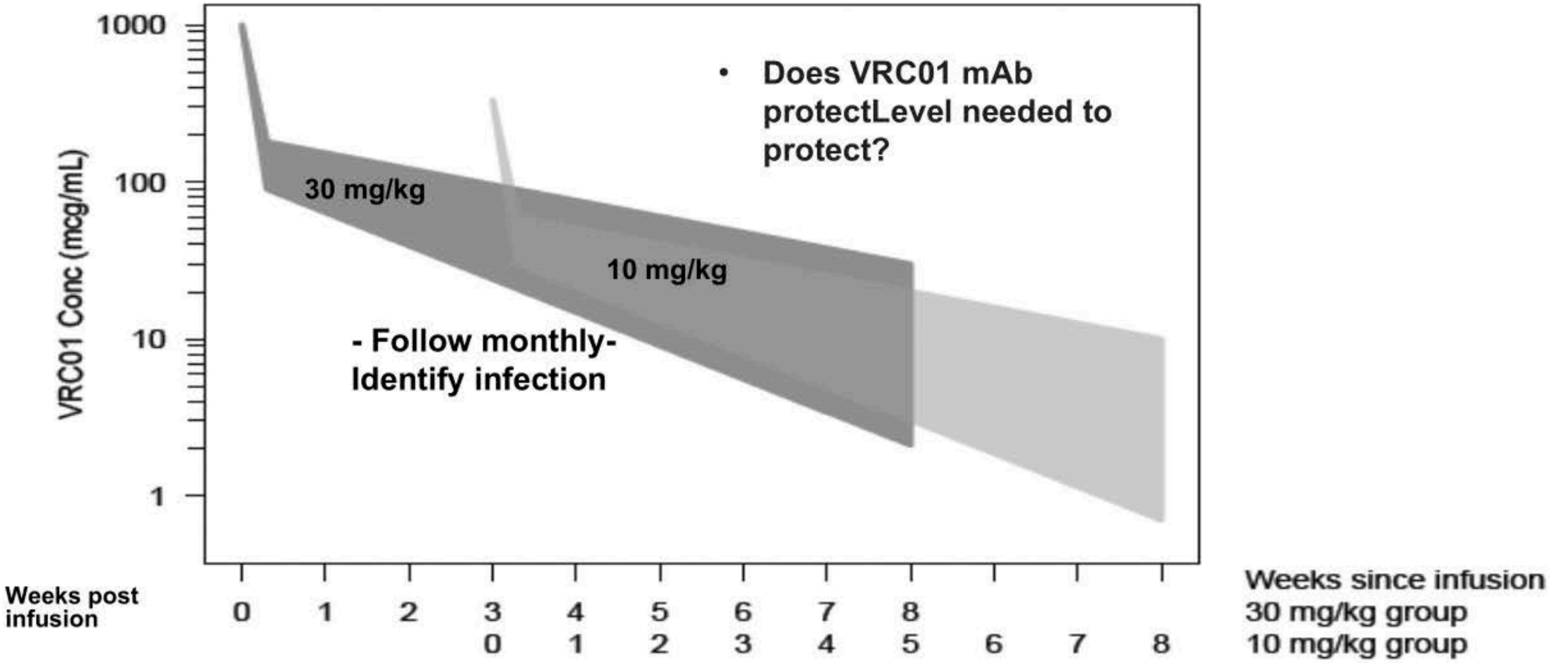
DAIDS

Associate Serum mAb Level with Time of Infection



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- VRC01 administered at 30 mg/kg, or 10 mg/kg, vs placeboAdministered every 8 weeks by IV infusion

What Happens With Success:
i.e. If this antibody decreases risk of infection

- Learn how much antibody (serum level) is needed to protectLearn if viral resistance is important

- IF AN ANTIBODY WORKSIncentive to fully develop the next generation mAbs: more potent and long actingIncentive for commercial development of HIV mAbsImplications for vaccine development

SQ route once every 3-4 months

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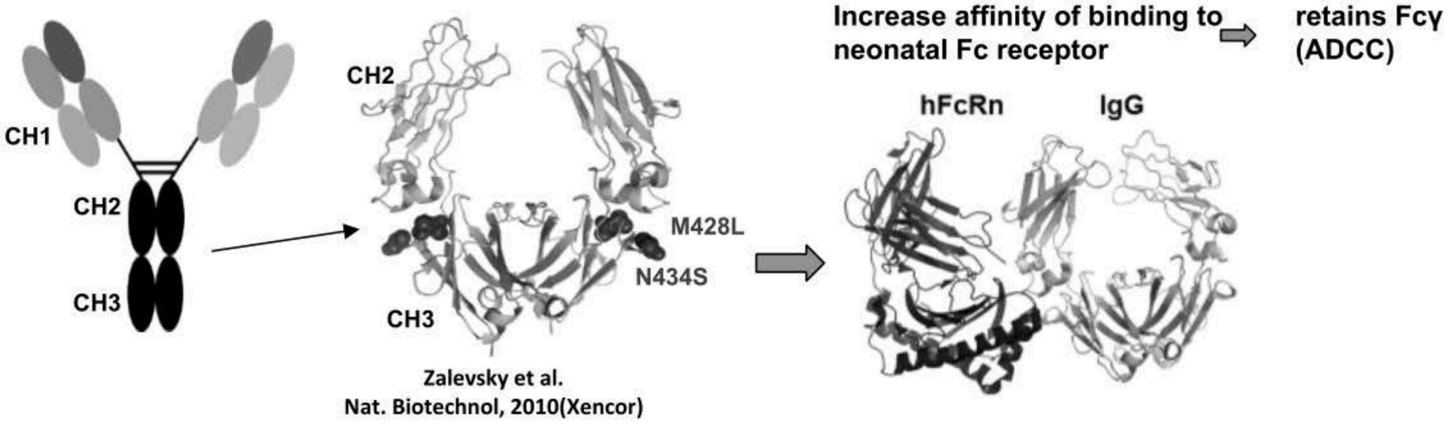
n – increased affinity for FcRn:Prolongs circulat



- Fc region binds with high affinity to FcRn at acidic pH (<6.5) in endosomeProtects antibody from endosomal degradation IgG released back into circulation at physiological pH (7.4)Results in prolonged circulating half life

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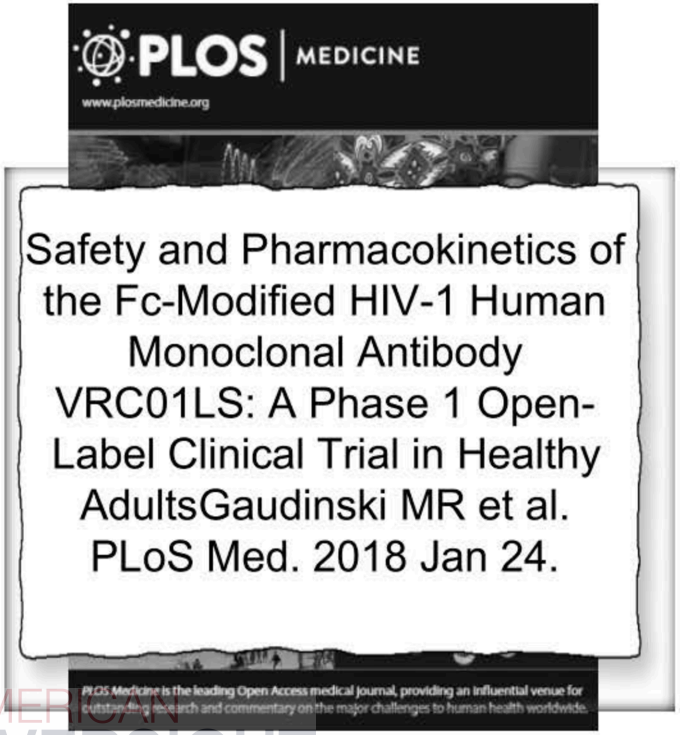
VRC01LS Phase 1 Trial Summary
(VRC 606)



- VRC01LS safe and well-tolerated when delivered IV or SCNo serious adverse events or dose-limiting toxicitiesVRC01LS retained its expected neutralizing activity in serum and anti-VRC01 antibody responses were not detected T ½ is 71 days (>4 fold longer than VRC01)

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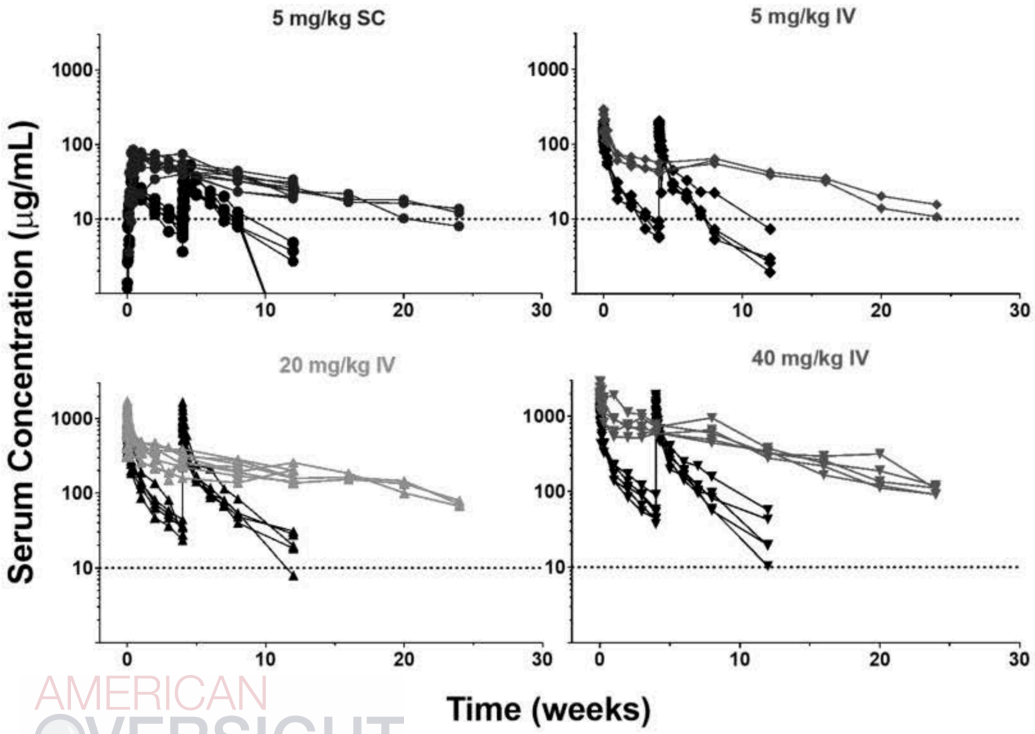
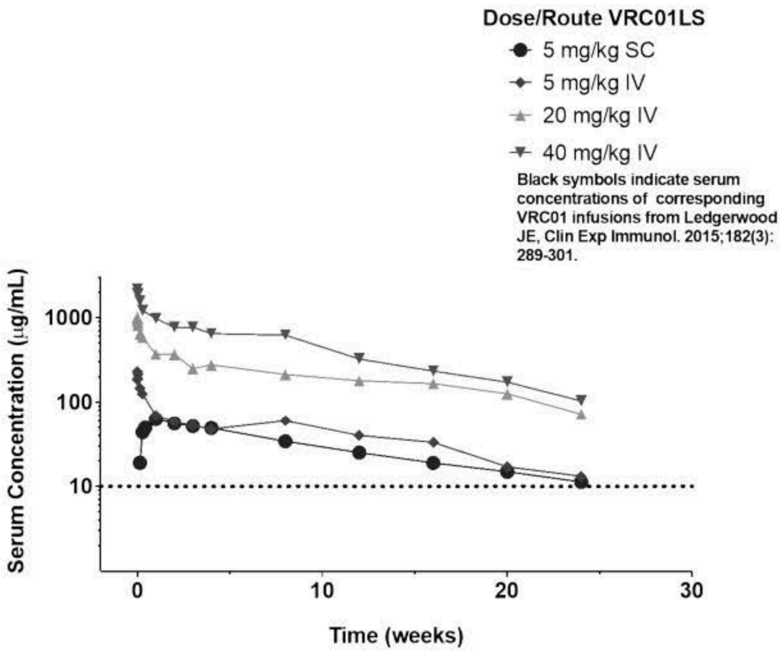
Serum Antibody Concentration (mcg/ml) of VRC01 and VRC01LS Following Single Administrations of VRC01LS





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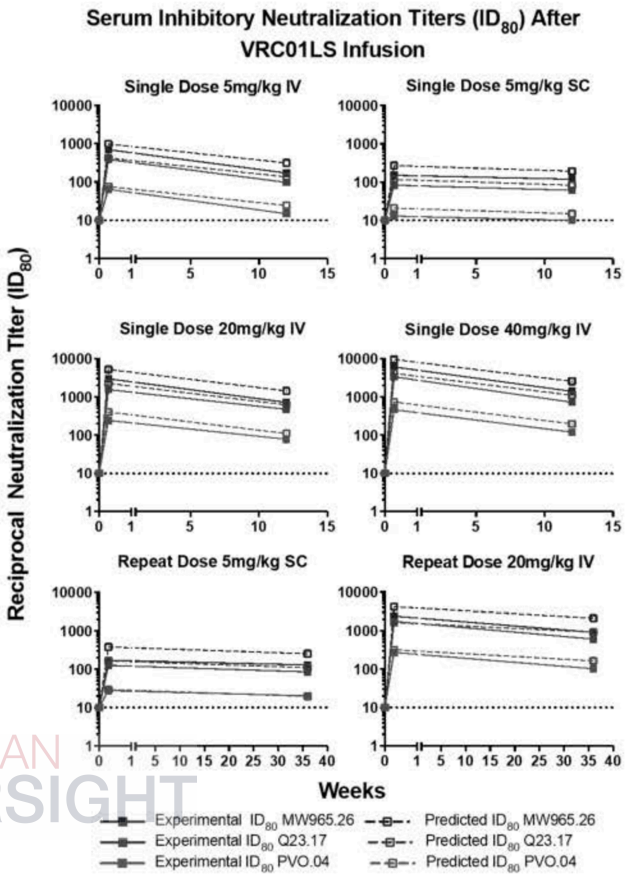
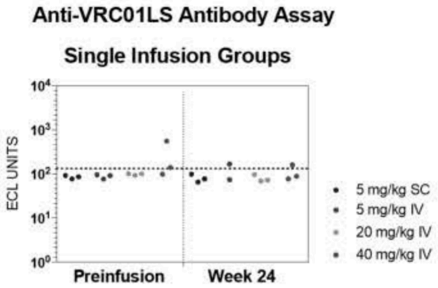
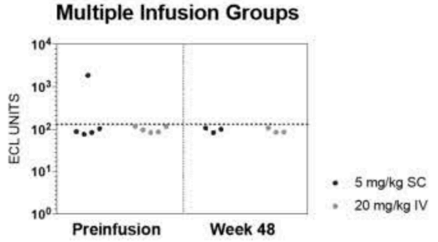
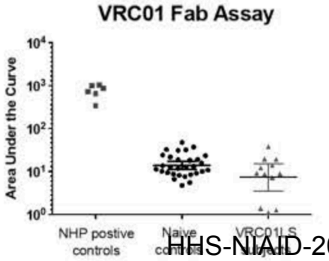
VRC01LS Post Infusion Serum Neutralization was Retained and no Antidrug Antibody (ADA) was Detected









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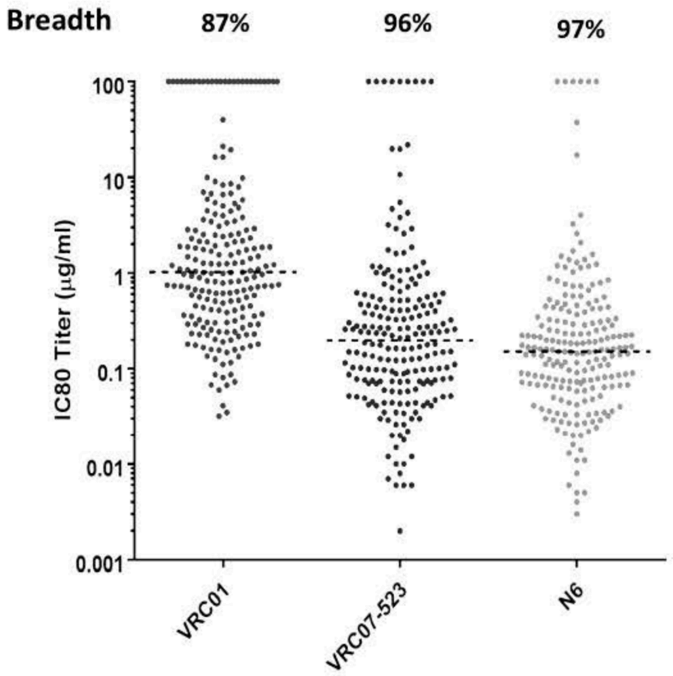
Next Gen Products:Improved Neutralization(VRC07523 and N258.N6)



- 10 fold more potent than VRC01Coverage improves to > 95%,VRC07523LS Phase 1 enrolledN6LS Phase 1 Spring 2018

Panel of 206 Env-pseudoviruses: Doria-Rose, Louder, McKee, Bailer et al.

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VRC07523LS Phase 1 Fully Enrolled

(VRC 605)

- Phase 1, Dose-Escalation, Safety and PKVRC07-523LS Administered IV or SC to Healthy AdultsAdministration (dose/route): Single dose of 1 mg/kg, 5 mg/kg, 20 mg/kg, 40 mg/kg IV, or 5 mg/kg SCRepeat doses of 20 mg/kg IV, or 5 mg/kg SC, at 12 week intervals

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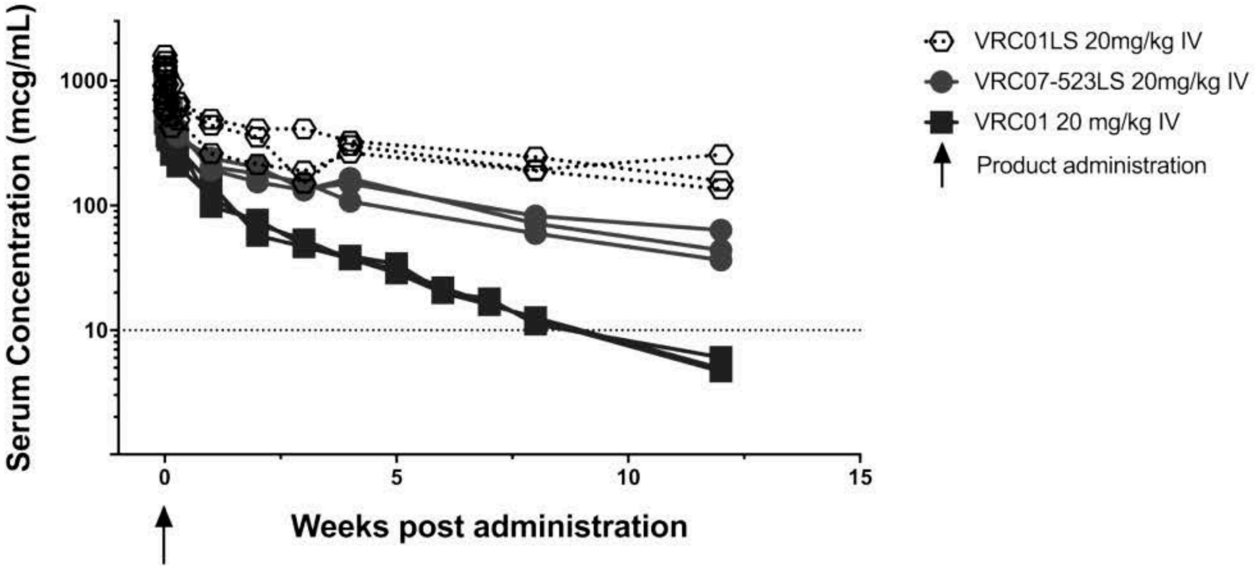
Subject serum concentration (mcg/mL) after a single administration of VRC07-523LS, VRC01LS, or VRC01



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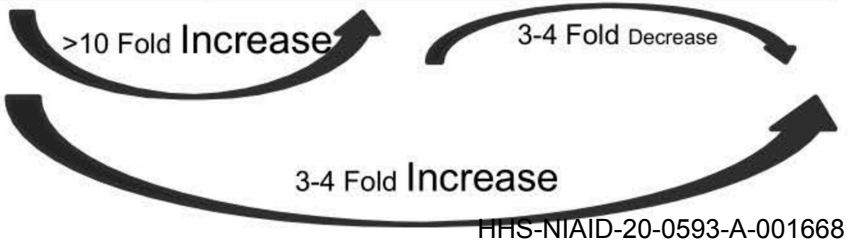
Serum Concentration by Dose 12 Weeks After Single Administration of VRC01, VRC01LS, and VRC07523LS

Serum Antibody Concentrations (mcg/mL)Group arithmetic mean (SD)			
Dose/Route(Single administration: week 0)	VRC01	VRC01LS	VRC07523LS
5 mg/kg IV	3.0 (2.7)	40 (2.7)	12 (1.8)
5 mg/kg SC	2.3 (2.1)	25 (1.7)	6.7 (1.4)
20 mg/kg IV	15 (11)	183 (64)	45 (12)
40 mg/kg IV	30 (19)	326 (35)	85 (30)



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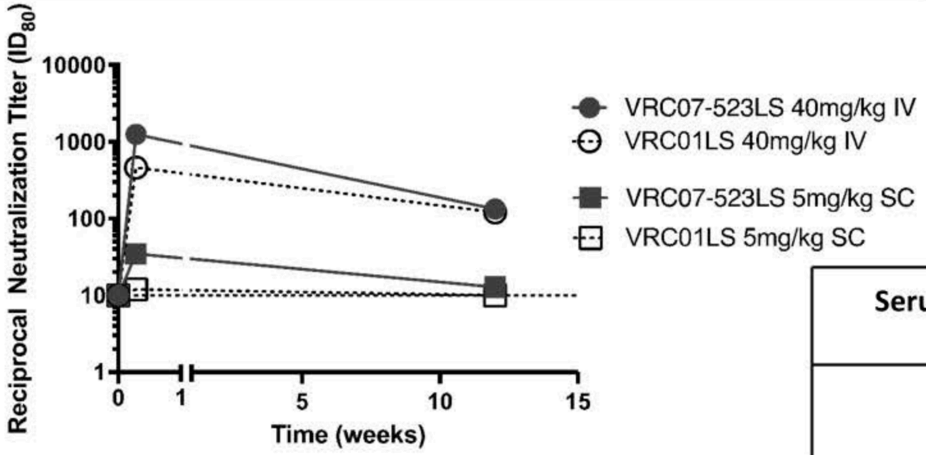
Neutralization (ID_{80}) of virus PVO.04 (clade B) for VRC07-523LS compared to VRC01LS



Serum concentration and potency against PVO.04 of VRC07-523LS and VRC01LS at 12 weeks post administration				
	Serum Concentration mcg/mL (SD)		Neutralization titer ID80 (SD)	
Antibody	5mg/kg SC	40mg/kg IV	5mg/kg SC	40mg/kg IV
VRC07-523LS	6.7 (1.4), n=7	85 (30), n=3	13 (5.0), n=3	133 (61), n=3
VRC01LS	26 (7.8), n=18	326 (35), n=5	10 (0), n=3	120 (20), n=5
Fold difference (VRC07-523LS/VRC01LS)	0.25	0.26	1.29	1.11

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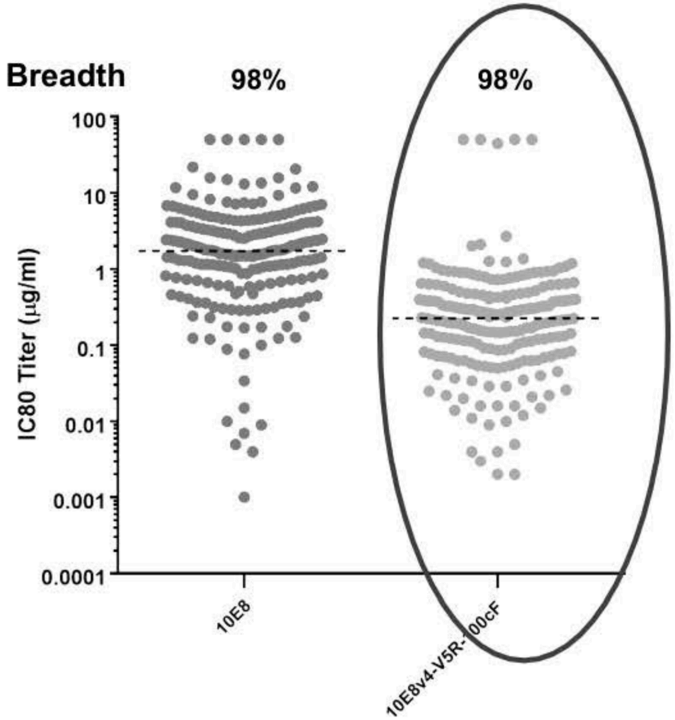
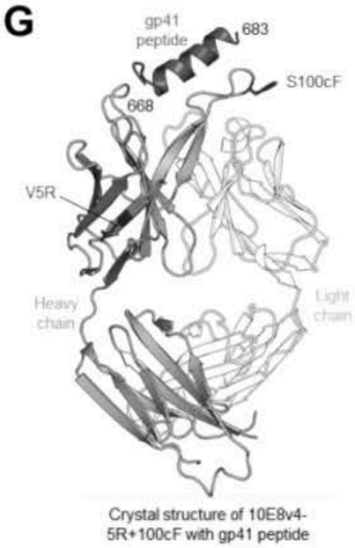
Improved Neutralization of 10e8 variantMPER mAb Clinical Product





Engineered: 10e8v5R:10-fold more potent than parental 10e8(Kwong et al, submitted)Added LS mutation=10e8VLSProduct is manufacturedPhase I in mid 2018

Multiclade Virus Panel (n=208) Doria-Rose, Louder, Bailer et al (VRC)

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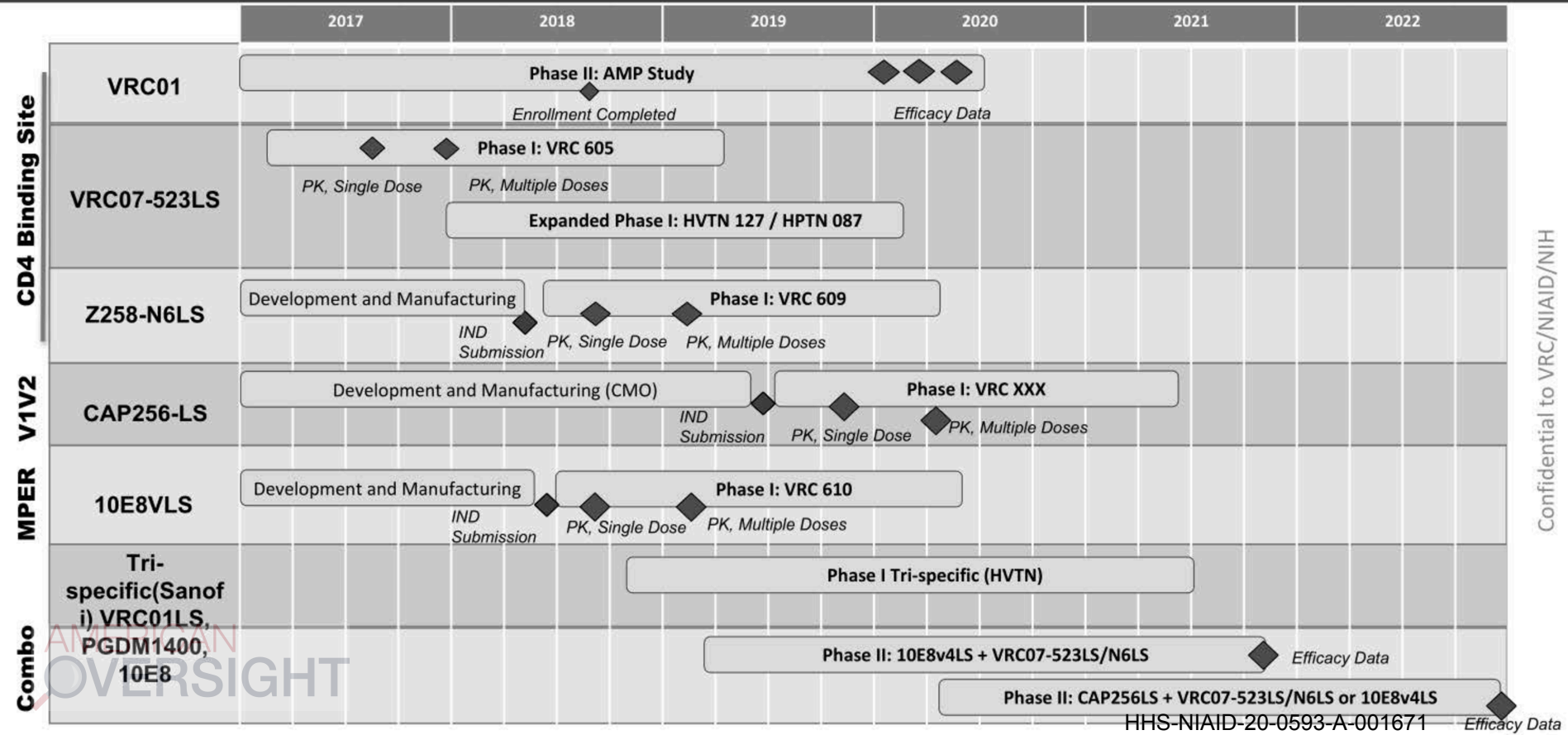
VRC HIV mAb Portfolio



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Summary

- VRC01 AMP efficacy trial will complete enrollment in 2018, data in 2019VRC01LS improves durability compared to VRC01 (>8-fold)VRC07523LS improves durability, neutralization and breadth over VRC0110E8VLS and N6LS will enter Phase 1 testing in mid 2018Combination studies to enter Phase 1/1b testing in 2018

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Acknowledgements

VRC Clinical Trials Program
Preeti Apte Alison BeckNancy Rebecca LampleyBrenda BerkowitzEugeania BurchLarkin Lam LeFloreliz Burgos FlorezCristina MendozaLaura NovikMark CarterGrace ChenEmily O'Callahan Abidemi CoatesPam CostnerJosephine Olalis PittmanSarah PlummerRo CoxJennifer CunninghamAlyssa RothwellJamie SaundersEllie EshunMartin GaudinskiIngelisa SebaSandra SitarStephanie GordonCarmencita TaylorCora Trelles Cartagena GravesMercy GuechCynthia Olga Starr Hendel Somia TrofymenkoOlga HickmanRenunda HicksLyudmyla VasilenkoXiaolin WangWil HolmanKate Houser WhalenPernell Williams Galina Yamshchikov

VRC John MascolaBarney GrahamRick KoupPeter KwongRobert BailerDanny DouekNicole Doria-RoseAmarendra PeguSandeep NarpalaMark LouderSijy O'DellRebecca Lynch Eli BoritzAdrian McDermottDavid CarltonAbe MittelmanMarybeth DaucherLucio GamaJason GallGary NabelRebecca Ruckly

VRC Clinical/RegulatoryCarrie LaurencotMichelle Conan-Cibotti Flo KaltovichJudy SteinVRC Product DevelopmentFrank ArnoldRichard Schwartz David LindsayGretchen SCheiberNIAID, BiostatisticsZonghui HuPK AnalysisEdmund CapparelliCAVD, VIMCM, SeamanD. Montefiori

HVTN, HPTNHVTN 104 and AMP study teams
Mike Cohen
Larry CoreyShelly KarunaKen MayerNIAIDCarl DieffenbachMary Marovich
Sarah ReadSheryl ZwerskiDiana FinziRandy TresslerMary AllenMarga GomezStephen MiguelesMark Connors

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National Institute of Allergy and Infectious Diseases
Vaccine Research Center

Vaccines for Life™

Extra slides

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Potency

Reference: IC_{80} values for VRC01, VRC01LS, and VRC07523LS

Virus strain	Clade	VRC07-523LS	VRC01LS	VRC01	VRC07-523LS vs VRC01fold higher potency
CNE59	AE	0.477	3.14	3.86	8.1
DU156.12	C	0.116	0.392	0.482	4.2
MW965.26	C	0.048	0.149	0.134	2.8
PVO.04	B	0.460	1.431	1.666	3.6
Q23.17	A	0.170	0.209	0.237	1.4
THRO.18	B	6.93	21.4	20.1	2.9
ZM53.12	C	1.17	4.09	5.06	4.3
6322.V4.C1	C	0.135	>50.0	>50.0	>370
CAP210	C	8.63	>50.0	>50.0	>5.8
MuLV	-	>50.0	>50.0	>50.0	NA

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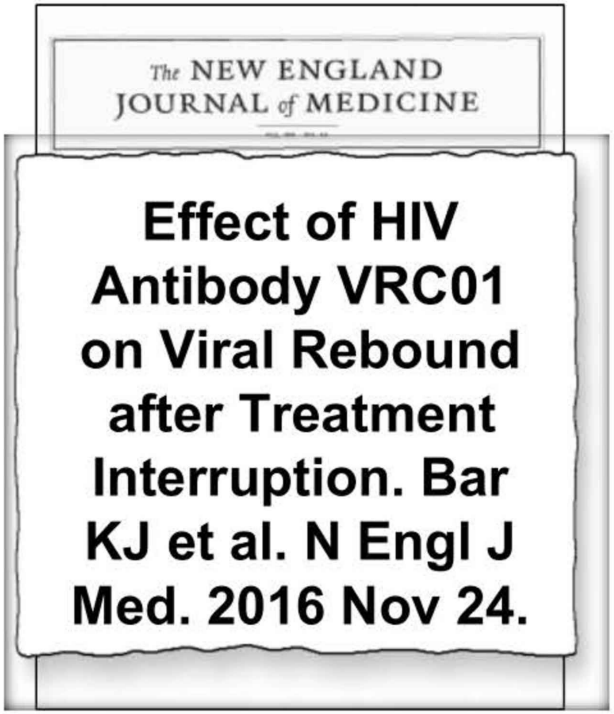
VRC01 Assessed in HIV ATI Clinical Trials

- VRC01 was safe and slightly delayed plasma viral rebound in 2 small ATI trialsVRC01 monotherapy did not maintain viral suppression by week 8 and combination therapy will be needed for a treatment approach



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VRC01LS Mean Pharmacokinetic Parameter Values

Group and dose	C_{max}	T_{max}	CL	$t_{1/2\beta}$	AUC	4 week post-infusion conc.	12 week post-infusion conc.
				Mean (s.d)			
Intravenous Dosing							
5 mg/kg (n=3)	246 (78)	0.07 (0.08)	40 (7)	83 (11)	4896 (499)*	48 (8)	40 (2.7)*
20 mg/kg (n=8)	1221 (397)	0.2 (0.3)	33 (8)	76 (19)	23368 (5279)*	276 (71)	180 (43)*
40 mg/kg (n=5)	2234 (548)	0.05 (0.02)	38 (9)	55(7)	57099 (13679)	651 (86)	326 (35)
Overall IV (n=16)			36 (8)	71 (18)			
Subcutaneous Dosing							
5 mg/kg (n=9)	69 (15)	9.0 (7.9)	61 (5)+	66 (24)	3777 (814)	49 (11)	25 (5.3)

Includes PK parameters from volunteers who received one or more doses of VRC01LS.* AUC and week 12 values are missing one volunteer from the overall group due to missing week 12 PK samples.+ Value following SC administration represents CL/F.AUC, area under the curve, 0±12 weeks (µg × day/mL); CL, clearance (mL/day); CL/F, apparent clearance; Cmax, maximal serum concentration (µg/mL); Tmax, time to maximal concentration (days); t1/2β, elimination half-life(days)

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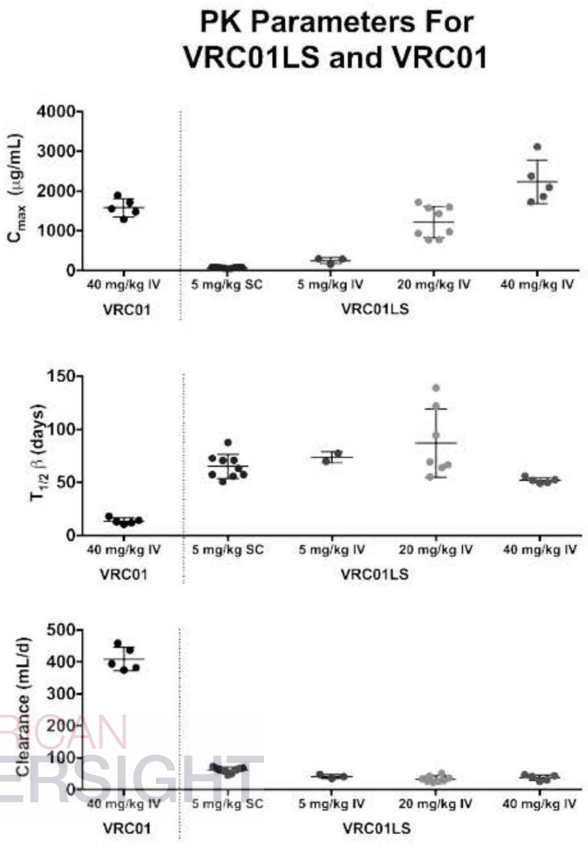
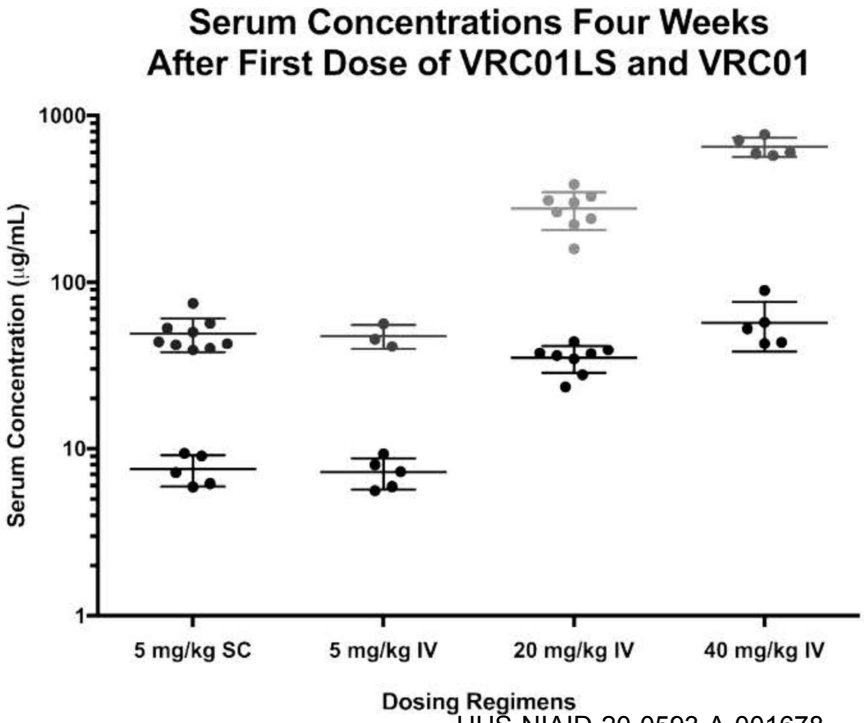
VRC01 and VRC01LS: PK Parameters and Post Infusion Serum Antibody Concentration





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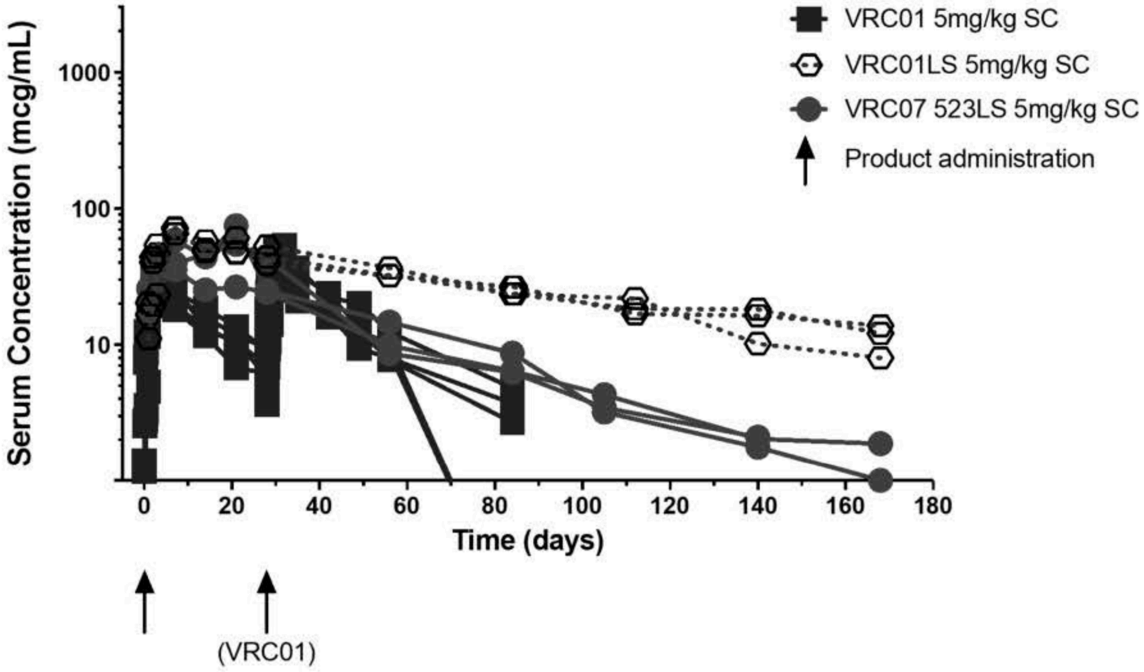
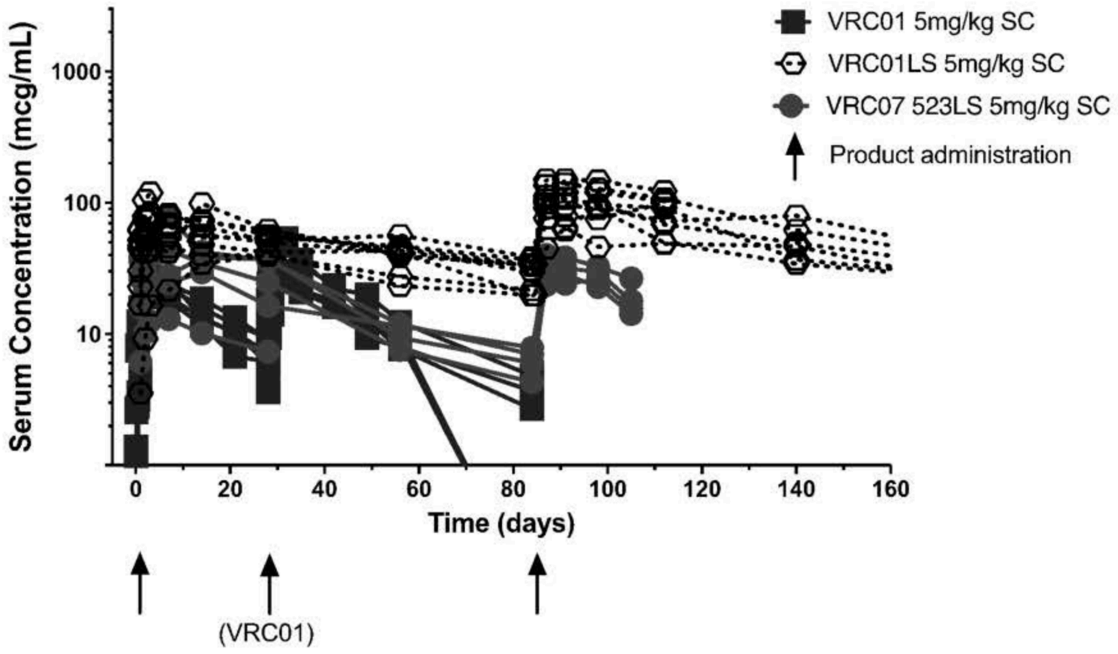
Antibody serum concentration (mcg/ml) of VRC01, VRC01LS, and VRC07-523LS at 5 mg/kg SC after a single administration



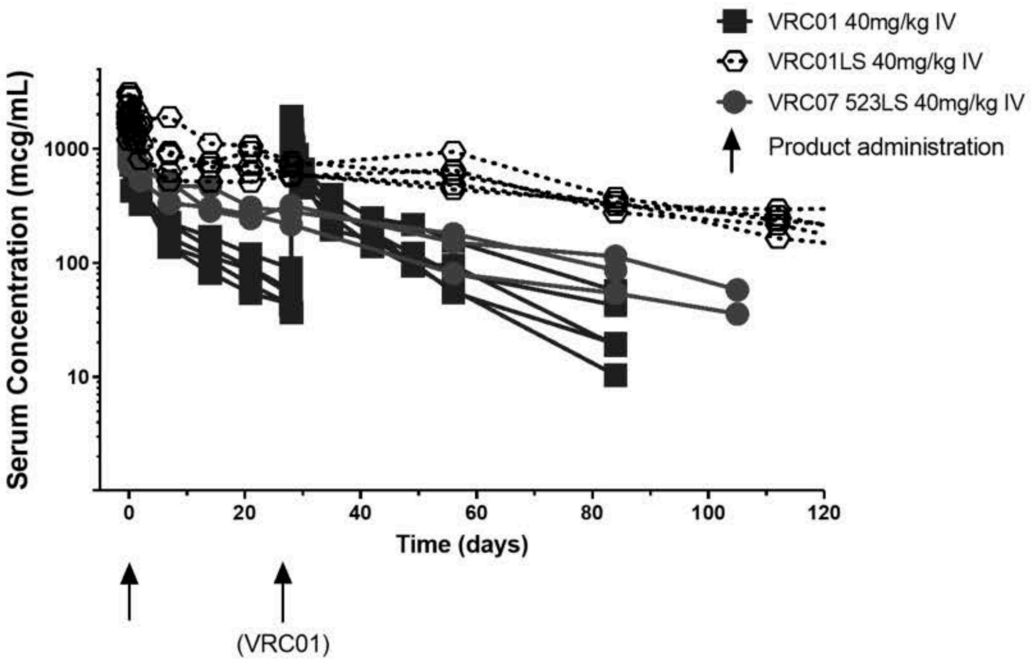
AMERICAN OVERSIGHT
CONFIDENTIAL Interim and Preliminary

Antibody serum concentration (mcg/ml) of VRC01, VRC01LS, and VRC07-523LS at 5 mg/kg SC after multiple admins



AMERICAN OVERSIGHT
CONFIDENTIAL Interim and Preliminary

Antibody serum concentration (mcg/ml) of VRC01, VRC01LS, and VRC07-523LS at 40 mg/kg IV



AMERICAN OVERSIGHT
CONFIDENTIAL Interim and Preliminary

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Tue, 24 Apr 2018 11:52:26 +0000
To: Auchincloss, Hugh (NIH/NIAID) [C] (b)(6)
Subject: FW: OS Clearance Request - Due by 9am Monday April 23rd`
Attachments: 1 RFA-GH18-1855.docx

Please take a look at this and take care of it.
Thanks,
Tony

From: Lauer, Michael (NIH/OD) [E]
Sent: Monday, April 23, 2018 6:37 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6) Auchincloss, Hugh (NIH/NIAID) [E] (b)(6)
Cc: Fenton, Matthew (NIH/NIAID) [E] (b)(6) Lauer, Michael (NIH/OD) [E] (b)(6)
Subject: FW: OS Clearance Request - Due by 9am Monday April 23rd`

Hi Tony and Hugh – you probably already know about this, but just in case, FYI.

Thanks, Mike

From: "Brandy, Aesha (NIH/OD) [C]" (b)(6)
Date: Friday, April 20, 2018 at 3:13 PM
To: "Lauer, Michael (NIH/OD) [E]" (b)(6) "Black, Jodi (NIH/OD) [E]" (b)(6)
Cc: "Bundesen, Liza (NIH/OD) [E]" (b)(6) "Kosub, David (NIH/OD) [E]" (b)(6) "Joshi, Pritty (NIH/OD) [E]" (b)(6) "Showe, Melanie (NIH/OD) [E]" (b)(6) "Kitt, Cheryl (NIH/OD) [E]" (b)(6)
Subject: OS Clearance Request - Due by 9am Monday April 23rd`

Hi Dr. Lauer and Dr. Black –
Please see the attached "New Notice of Funding Opportunity: Partnership with China CDC on Infectious Disease Prevention and Control in China and Globally" assigned from the Office of the Secretary that OER is being asked to review and clear. Would you mind reviewing and providing your concurrence and/or comments by 9am Monday, April 23rd?

Apologies for the short turnaround – this is due to the OS deadline. Please let me know if you have any questions or feels this should be assigned to another SME.
Thank you and have a good weekend.

Best Regards,
Aesha Brandy, MBA*
Program Analyst
NIH/OER/IMOD

AMERICAN OVERSIGHT

Building 1, Room 138
Bethesda, MD 20814
(b)(6)
(b)(6)
*Master Key Consulting Contractor

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Funding Opportunity Number: CDC-RFA-GH18-1855

Funding Opportunity Title: Partnership with China CDC on Infectious Disease Prevention and Control in China and Globally

Program Office: Center for Global Health

Drafter: Randi Daniels

Special Instructions:

Gray shaded text denotes standardized language that is required content in every announcement.

Gray shaded underlined text denotes optional standard language where users can opt to include certain text when appropriate.

Part I. Overview Information

Applicants must go to the synopsis page of this announcement at www.grants.gov and click on the "Send Me Change Notifications Emails" link to ensure they receive notifications of any changes to CDC-RFA-GH18-1855. Applicants also must provide an e-mail address to www.grants.gov to receive notifications of changes.

A. Federal Agency Name:

Centers for Disease Control and Prevention (CDC) / Agency for Toxic Substances and Disease Registry (ATSDR)

B. Notice of Funding Opportunity (NOFO) Title:

Partnership with China CDC on Infectious Disease Prevention and Control in China and Globally

C. Announcement Type: New - Type 1

This announcement is only for non-research activities supported by CDC. If research is proposed, the application will not be considered. For this purpose, research is defined at https://www.gpo.gov/fdsys/pkg/CFR-2007-title42-vol1/pdf/CFR-2007-title42-vol1-sec52-2.pdf. Guidance on how CDC interprets the definition of research in the context of public health can be found at https://www.hhs.gov/ohrp/regulations-and-policy/regulations/45-cfr-46/index.html (See section 45 CFR 46.102(d)).

D. Agency Notice of Funding Opportunity Number:

CDC-RFA-GH18-1855

E. Catalog of Federal Domestic Assistance (CFDA) Number:

93.318

F. Dates:

1. Due Date for Letter of Intent (LOI): **N/A**

2. Due Date for Applications: **06/15/2018**, 11:59 p.m. U.S. Eastern Standard Time, at www.grants.gov.

3. Date for Informational Conference Call:

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HHS-NIAID-20-0593-A-001684

N/A

G. Executive Summary:

1. Summary Paragraph:

The purpose of this 5-year project is to continue to collaborate with the government of China to reduce the threat of infectious disease transmission in China and around the world, which in turn contributes to the common defense of both countries against emerging and pandemic threats and strengthens our bilateral relationship with China in public health. The program has three broad goals: 1) to jointly carry out public health activities to prevent, detect, and respond to emerging and re-emerging infectious diseases and public health emergencies in China that have potential for global impact, 2) to further develop China's own capacity to carry out these public health activities, and 3) to enhance China's role as a global partner to support public health strengthening of other countries. Activities may include joint investigating and monitoring of disease outbreaks, piloting innovative public health interventions, consulting on outbreak responses and risk communication, strengthening public health capacity, strengthening technical partnerships, promoting rapid and transparent dissemination of public health data in China, helping develop China CDC's Center for Global Public Health, jointly supporting the Africa CDC, and collaborating on and coordinating global public health activities in other countries. Depending on funding availability and priorities, the specific infectious disease entities to be addressed are expected change over the period of performance.

a. Eligible Applicants: Single

b. NOFO Type: Cooperative Agreement

c. Approximate Number of Awards: 1

d. Total Period of Performance Funding: $25,000,000

e. Average One Year Award Amount: $5,000,000

This amount is subject to the availability of funds.

f. Total Period of Performance Length: 5

g. Estimated Award Date: 09/30/2018

h. Cost Sharing and / or Matching Requirements: N

Part II. Full Text

A. Funding Opportunity Description

Part II. Full Text

1. Background

a. Overview

For the past 30 years, CDC has collaborated with China on public health priorities that affect China, the US, and the world. This collaboration has helped build the public health system in China, address health issues of importance to both countries, and build a strong relationship

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between the US and China on public health. However, as China's capacity, health status, and global role grow, the nature of the collaboration is evolving from one of assistance to one of partnership. Nonetheless, China still needs specialized technical support to continue developing its public health system while also contributing to improving public health globally.

A disease threat anywhere is a disease threat everywhere; moreover, disease outbreaks can devastate economies, destabilize political environments, and impact national security. China is at special risk for outbreaks of emerging and re-emerging infections, in part because its large population of both people and livestock result in significant human-animal interface and increased spread of zoonotic disease. China has the 3rd highest burden of tuberculosis (TB) globally and increasing antibiotic resistance. Moreover, China is linked to the world through expanding trade and air travel, increasing the risk for infections spreading from China to the US and the rest of the world. Thus, it is in the interest of both the US and China to collaborate to reduce the threat of infectious diseases in China that have potential for spread globally.

CDC has been partnering with China to build China's public health capacity—strengthening public health surveillance, preventing disease transmission, and improving laboratory detection of infectious diseases, such as influenza, acute respiratory infections, food-borne infections, vaccine-preventable diseases, HIV, and drug-resistant TB. CDC has supported the Chinese Field Epidemiology Training Program to strengthen disease surveillance, epidemiology, and capacity to respond to infectious disease and other public health emergencies in China and to develop future public health leaders. CDC has advised China on science-based communication strategies to mitigate public health threats and address public health priorities. Whereas these efforts have greatly strengthened China's public health capacity, advanced technical support is needed to help China continue to develop into a global public health leader.

Since most of the world remains under-prepared to effectively prevent, detect, and respond to disease threats, helping China build its capacity to assist other countries benefits the US, China, and the world. In response to the 2014 Ebola outbreak in West Africa, China demonstrated its growing capacity to improve public health systems in other countries. This shift has resulted in China and US commitments to close collaboration on global health, such as jointly supporting Africa CDC, and helping China continue to build capacity in global health.

In 2017, the Presidents of US and China agreed to carry out joint activities to control infectious diseases. Continuing close relationships between the US and China built through decades of public health collaboration provide a foundation essential to work together to respond to future global pandemics and other public health emergencies that threaten Americans at home and abroad. The purpose of this cooperative agreement is for CDC to continue to collaborate with China to reduce the threat of infectious diseases in China and around the world, which in turn contributes to the common defense of both countries against health threats.

b. Statutory Authorities

Public Health Service Act, Sections 307 and 301(a) of the Public Health Service Act [42 USC242l; 42 USC 241(a)]

c. Healthy People 2020

Healthy People 2020 (https://www.healthypeople.gov/2020/topics-objectives/topic/global-health/objectives) goals supported by this NOFO include: GH-5 - Increase diagnostic testing capacity in

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host countries and regionally through the Global Disease Detection Regional (GDD) Centers.

d. Other National Public Health Priorities and Strategies

The Department of Health and Human Services' (HHS) Global Health Strategy: (https://www.hhs.gov/about/agencies/oga/about-oga/why-hhs-works-globally/hhs-global-strategy/index.html).

National Action Plan (NAP) for Combating Multidrug-Resistant Tuberculosis (MDR TB): (https://obamawhitehouse.archives.gov/sites/default/files/microsites/ostp/national_action_plan_for_tuberculosis_20151204_final.pdf)

Commitment by US President Donald J Trump and China President Xi Jinping to carry out joint projects on infectious diseases (https://www.whitehouse.gov/briefings-statements/president-donald-j-trumps-state-visit-china/).

e. Relevant Work

This NOFO builds upon the long-term relationship between US CDC and China CDC, as two major country National Public Health Institutes. An important area of this collaboration over the past decade has been in the area of infectious disease prevention and control. This NOFO also builds on specific collaborations with China CDC in HIV, influenza, TB, vaccine-preventable diseases, and global health. In particular this NOFO builds on and expands previous non-research NOFO CDC-RFA-GH12-1247, and current research cooperative agreement RFA-GH-17-005.

2. CDC Project Description

a. Approach

Bold indicates period of performance outcome.

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i. Purpose

The purpose of this funding opportunity is to continue collaborating with the government of China to promote the common defense of both countries against emerging and pandemic infectious disease threats. This will be done by 1) jointly carrying out public health activities to prevent, detect, and respond to emerging and re-emerging infectious diseases in China that have potential for global impact, 2) developing China's own capacity to carry out these public health activities, and 3) enhancing China's role as a global partner to support public health strengthening of other countries.

ii. Outcomes

- Outcomes of this project include strong public health surveillance and emergency response systems capable of preventing, detecting, and responding to infectious disease threats in China and other countries; effective public health response to infectious disease emergencies that arise in China and other countries; sustained reduction in threat of infectious diseases in US, China, and other countries; and long-term public health impact through sustained partnership between the US and China that contributes to the common defense of both countries.

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- The long-term impact of this effort will be healthier people in China, US, and the world.

iii. Strategies and Activities

This NOFO supports activities directed at the prevention and control of infectious diseases that have potential for global impact, including emerging and re-emerging infectious diseases such as influenza, tuberculosis, vaccine-preventable diseases, malaria, drug-resistant infections, bacterial respiratory diseases, enteric diseases, acute febrile illnesses, and other infectious diseases. These activities may include the conduct of public health surveillance; epidemiological assessments, outbreak response, development of high quality laboratory systems and diagnostic capacity, strengthening the timely and transparent sharing of public health information, and implementation of innovative public health disease control programs.

It also will support the provision of training opportunities for public health professionals, including laboratory personnel, China CDC staff, university and select graduate level students and other program implementers in health assessment and epidemiology, program planning, program implementation, program evaluation, data collection and analysis as well as personnel, financial and administrative management. This training will support public health efforts in China as well as those China may carry out in other countries.

To achieve the goals and intended outcomes of this program, possible recipient activities during the period of performance may include the following:

- Improve infectious disease surveillance, lab diagnosis, and emergency response capacity, and develop skilled public health workforce
- Maintain and strengthen technical partnerships with China's provinces, policymakers, academia, and clinicians
- Strengthen rapid dissemination of data to the public health community to expedite public health response, including through a public health bulletin
- Jointly monitor outbreaks
- Pilot innovative interventions
- Consult on outbreak preparedness, response, and communication strategies
- Develop the capacity of China CDC's Center for Global Public Health
- Jointly carry out capacity building activities to support Africa CDC
- Collaborate and coordinate on global health activities in other countries
- Build capacity in TB infection control, surveillance, and laboratory practices to strengthen TB, including MDR TB prevention and control efforts
- Collaborate on activities to improve and innovate MDR TB diagnosis and linkage to care
- Strengthen the role of public health in preventing health care- associated infections, and antimicrobial resistance, especially with TB

1. Collaborations

a. With other CDC programs and CDC-funded organizations:

Recipient is expected to collaborate with various CDC Divisions and Programs to ensure that the

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activities and materials meet CDC scientific and ethical standards, maximize resources, avoid duplication, and concur with CDC published recommendations and disease specific information. Specifically, recipient is expected to collaborate with the Center for Global Health and other divisions across CDC that will provide technical and financial support to projects within the cooperative agreement. Expected collaborations include the Division of Global HIV and TB (DGHT), Division of Global Health Protection (DGHP), Influenza Division, and others, including US CDC field offices in other countries when appropriate. Recipient is also expected to collaborate as appropriate with other CDC-funded organizations, such as WHO and Africa CDC. It is expected that the recipient will engage with internal and external stakeholders (noted below) who contribute to the goals identified in the announcement during development and implementation of activities and the evaluation performance plan.

b. With organizations not funded by CDC:

Collaborations with organizations not funded by CDC may occur across all levels of China CDC (national, provincial, local). Recipient is expected to collaborate with key partners including the National Health Commission, local universities, and other local and international civil societies who are working in China. Other collaborations outside China may include with Ministries of Health of other countries, and other regional and international public health organizations. Recipient should work closely with all stakeholders in the finalization of the evaluation performance measurement plan (later defined in this announcement) including China CDC program managers, service delivery stakeholders (e.g., hospital health care providers, laboratory technicians, peer counselors), and other technical assistance stakeholders.

2. Target Populations

This announcement is designed broadly to have health impact on reducing the spread of infectious diseases in China and around the world, including the US, through partnership between US CDC and China CDC on activities in China and other countries. Specific target populations will vary depending on programmatic area and specific activities. Applicant should state in their funding requests whether and how target populations will be addressed.

a. Health Disparities

By reducing infectious disease threats across the whole population of China and in other countries, this project especially helps reduce risk of infectious diseases among under-served populations. Applicant should describe how they will ensure that underserved populations are included in project planning and implementation.

iv. Funding Strategy

Multiple programs may be contributing to this award, and there may be multiple funding streams available for different strategies and activities. The funding strategy is designed to be flexible to allow for new priorities that may emerge over the course of the period of performance. The maximum total funding (direct and indirect) for the first year (12 month budget period) is 5,000,000; and the maximum total funding for the 5-year period of performance is 25,000,000. Actual funding amounts may be less. Awards issued under this NOFO are contingent upon the availability of funds.

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b. Evaluation and Performance Measurement

i. CDC Evaluation and Performance Measurement Strategy

The purpose of evaluation and performance measurement is to help CDC recipients: (1) Monitor the extent to which activities were successfully completed (e.g., Were activities implemented correctly?); (2) Demonstrate progress toward achieving program outcomes (e.g., Were outcomes of interest achieved?); and (3) Inform decisions about future programming that drive continuous program improvement for more efficient and effective program performance (e.g., What and how could things be improved?).

An Evaluation and Performance Measurement Plan (EPMP) is required as part of this NOFO to measure NOFO accomplishments. The primary objective of the EPMP implementation is to demonstrate how activities under this NOFO related and lead to the desired outcomes. The results will be used for program improvement, accountability and reporting, and for sharing with partners and other stakeholders. The EPMP framework describes how CDC will work with the recipient on NOFO activities related to performance measurement and evaluation, recipient requirements, engagement of stakeholders, information dissemination, and allocation of funds for EPMP.

CDC Approaches for Performance Measurement:

Throughout the five-year NOFO period, CDC will work with the recipient to measure implementation performance. Note that CDC will:

- Use results of performance measures continuously to determine whether NOFO activities and outcomes were achieved as planned.
- Provide technical assistance to the recipient to support routine data collection, analysis of key performance indicators, and quarterly reporting to CDC.
- Review monitoring results and provide feedback, noting any necessary corrective and/or improvement actions, according to performance measurement standards.
- In partnership with the recipient, conduct regular site monitoring in order to (1) demonstrate accountability of the NOFO investments, and (2) prioritize quality improvement actions needed to achieve program accomplishment as planned.

CDC Approaches for NOFO Evaluations:

Throughout the five-year NOFO period, CDC will work with the recipient to conduct program evaluations in order to determine the effectiveness of the proposed activities under this NOFO. The evaluations will include process and outcome evaluations of the funded activities.

- *Process Evaluation:* Process evaluations address evaluation questions related to resources, operations, and implementation. Example process evaluations might examine expenditures or cost of implementing a specific program, the extent to which and how well the agreed NOFO activities have been implemented as planned, whether key participants in the program received training or support needed, and whether a program is achieving early outputs or outcomes. Limitations, barriers, and key success factors of implementation will be explored, including external factors that influenced program delivery. It is expected that this evaluation will be conducted during Years 2-3.

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- *Outcome Evaluation*: Outcome evaluations address evaluation questions that address benefits, changes, or results of a program. Outcome evaluations seek to determine whether the funded activities are leading to the expected outcomes and in what manner the target audience benefited from a program. Findings in an outcome evaluation should highlight programmatic best practices and lessons learned and identify recommendations for program scale up, adaptation, and sustainability. It is expected that this evaluation will be conducted during Years 3-4.

CDC will work with the recipient to develop and finalize relevant, feasible, and useful performance measures for both process and outcome evaluations. Illustrative suggestions for possible process and outcome measures for the activities and strategies described in the logic model include:

Proposed Process Measures:

1. Jointly monitor surveillance data during outbreak investigations and consult on outbreak response

    - Number of outbreak investigations with joint monitoring and consultation

2. Collaborate on innovative infectious disease control activities

    - Number of protocols developed and implemented for interventions
    - Population reached through intervention
    - Number of evaluations conducted for intervention and plans developed by China CDC for scaling up

3. Collaborate on building core public health capacities for disease control in China, including epidemiology, surveillance, laboratory, workforce, outbreak and emergency response, informatics, and public health communications

    - Number of surveillance protocols developed and implemented
    - Number of new epidemiologists trained through CFETP program, stratified by province and region
    - Number of animal health experts or veterinarians or vet techs trained who can address the human and animal health interface
    - Number of mentors receiving mentor training
    - Number of laboratories accredited.

4. Provide technical support for the development and growth of a public health bulletin

    - Successful launch and routine dissemination by China CDC of public health bulletin

5. Collaborate on building capacity in global public health to help control the spread of infectious diseases in other countries, and coordinating global health activities between the US and China

    - Number of joint trainings and other joint activities conducted by China CDC and US CDC in other countries
    - Number of trainings, personnel exchanges, workshops, and other activities to build

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capacity of China CDC Center for Global Public Health

6. Strengthen long-term partnerships between US and China in public health, including with national CDC, provincial CDCs, policy makers, academia, and clinicians that support the goals of the cooperative agreement

- Number of investigations jointly carried out between US and China CDC
- Number of projects carried out with technical and programmatic input from provincial CDCs, academia, and clinicians

7. Build capacity in TB infection control, surveillance, and laboratory practices to strengthen TB, including MDR TB, prevention and control efforts.

- Number of capacity building activities in TB infection control, surveillance and lab.

8. Collaborate on activities to improve and innovate MDR TB diagnosis and linkage to care.

- Number of collaborative activities on MDR TB

Proposed Outcome Measures

1. Effective public health response to infectious disease emergencies in China and other countries:

- Number of invitations received to assist in infectious disease emergencies in other countries
- Number of response teams and/or technical assistance teams within China
- Number of response teams and/or technical assistance teams sent to other countries
- Number of joint US-China global health activities completed
- Number of global health initiatives with substantial participation from China CDC

2. Reduced threat of infectious diseases in US, China, and other countries;

- Number of cross-border disease surveillance collaborations and information sharing
- Number of outbreak responses conducted by FETP trainees
- Time between detection and response to suspect outbreaks
- Time of reporting of information and potential public health emergencies to WHO

3. Improved public health capacity to prevent, detect, and respond to infectious disease threats in China and other countries; and

- Quality and timeliness of data collected (i.e. – completeness of reports) from baseline
- Number of surveillance programs for priority infectious diseases
- Number of provinces that meet WHO definition of 1 trained field epidemiologist/

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200,000 people in population
- Number of public health stakeholders (i.e. provincial CDCs) who receive national and sub-national surveillance data through a routinely published MMWR-like publication
- Number of CDC departments (at national, provincial, local levels) that have access to disease investigation and surveillance data for program decision making and response
- Number of policies and guidelines that incorporate results of surveillance and epidemiological information supported by this NOFO
- Successful achievement of laboratory accreditation

4. Long-term global health partnership between the US and China to support China's capacity to contribute to common defense of both countries.

- Number of publications in peer-reviewed publications for collaborative projects supported by this NOFO
- Number of annual US CDC – China CDC Directors meetings held

A system for collecting both quantitative and qualitative data over time to answer evaluation questions should be established during program implementation. To ensure that all evaluations are consistent with established frameworks and program specific requirements, submission of Non-research Determination (NRD) to CDC Associate Director for Science for ethical clearance is required. The CDC project officer will assist with the submission process.

The recipient will report evaluation findings to relevant stakeholders and make these publicly available in order to enhance the utilization of findings to improve program effectiveness, and continue program improvement upon completion of the award.

Timeframe:

Recipients will be required to submit a more detailed Evaluation and Performance Measurement Plan (EPMP), including a Data Management Plan (DMP), within the first 6 months of award, as described in the Reporting Section of this NOFO. Process evaluation will be conducted at the end of Year 2-3. The evaluation findings are expected to be reported by second quarter of Year 4. Outcome evaluation will be conducted at the end of Year 3-4. The evaluation findings are expected to be reported by second quarter of Year 5.

Dissemination of Results for Improved Program Quality and Results:

Dissemination of and feedback on performance measurements and evaluation results is an integral part of the quality improvement cycle and of the EPMP. Dissemination and feedback assists and encourages program managers and operational staff to make use of results when formulating, adapting, or scaling up activities. All stakeholders who are working on enhancing workforce capacity, improving quality of public health services and strengthening public health preparedness in China should be primary audiences for feedback and recommendations for program improvement and best practices, as well as lessons learned from the success and failure of activities. Audiences include national health policy makers, China CDC and other relevant NHFPC staff, representatives of UN agencies, and other multilateral health agencies. The recipient and other partners will use evaluation findings to improve program results by adjusting procedures to address barriers to successfully meeting public health goals identified in this award.

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In addition, the results, especially the Year 4 outcome evaluation will be a valuable tool for policy makers and health authorities for future policy and program planning.

ii. Applicant Evaluation and Performance Measurement Plan

Applicants must provide an evaluation and performance measurement plan that demonstrates how the recipient will fulfill the requirements described in the CDC Evaluation and Performance Measurement and Project Description sections of this NOFO. At a minimum, the plan must describe:

- How applicant will collect the performance measures, respond to the evaluation questions, and use evaluation findings for continuous program quality improvement.
- How key program partners will participate in the evaluation and performance measurement planning processes.
- Available data sources, feasibility of collecting appropriate evaluation and performance data, and other relevant data information (e.g., performance measures proposed by the applicant)
- Plans for updating the Data Management Plan (DMP), if applicable, for accuracy throughout the lifecycle of the project. The DMP should provide a description of the data that will be produced using these NOFO funds; access to data; data standards ensuring released data have documentation describing methods of collection, what the data represent, and data limitations; and archival and long-term data preservation plans. For more information about CDC's policy on the DMP, see https://www.cdc.gov/grants/additionalrequirements/ar-25.html.

Where the applicant chooses to, or is expected to, take on specific evaluation studies, they should be directed to:

- Describe the type of evaluations (i.e., process, outcome, or both).
- Describe key evaluation questions to be addressed by these evaluations.
- Describe other information (e.g., measures, data sources).

Recipients will be required to submit a more detailed Evaluation and Performance Measurement plan, including a DMP, within the first 6 months of award, as described in the Reporting Section of this NOFO.

Applicants must provide an evaluation and performance measurement plan that demonstrates how the recipient will fulfill the requirements described in the CDC Evaluation and Performance Measurement and Project Description sections of this NOFO. At a minimum, the plan must describe:

- How applicant will collect the performance measures, respond to the evaluation questions, and use evaluation findings for continuous program quality improvement.
- How key program partners will participate in the evaluation and performance

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measurement planning processes.

- Available data sources, feasibility of collecting appropriate evaluation and performance data, and other relevant data information (e.g., performance measures proposed by the applicant).
- Plans for updating the Data Management Plan (DMP), if applicable, for accuracy throughout the lifecycle of the project. The DMP should provide a description of the data that will be produced using these NOFO funds; access to data; data standards ensuring released data have documentation describing methods of collection, what the data represent, and data limitations; and archival and long-term data preservation plans. For more information about CDC's policy on the DMP, see https://www.cdc.gov/grants/additionalrequirements/ar-25.html.
- Where the applicant chooses to, or is expected to, take on specific evaluation studies, they should be directed to:
- Describe the type of evaluations (i.e., process, outcome, or both).
- Describe key evaluation questions to be addressed by these evaluations.
- Describe other information (e.g., measures, data sources).

Recipients will be required to submit a more detailed Evaluation and Performance Measurement plan, including a DMP, within the first 6 months of award, as described in the Reporting Section of this NOFO.

c. Organizational Capacity of Recipients to Implement the Approach

A successful applicant must demonstrate core organizational capacity to manage and conduct the activities for which awards are made. Applicants will demonstrate the local experience and institutional capacity (both management and technical) to achieve the goals of the NOFO with documented good governance practices. Applicant will demonstrate technical expertise in public health practice, including disease surveillance, workforce development, laboratory systems, and emergency response.

The application must include a clear plan for the administration and management of the proposed activities, and to manage the resources of the program, prepare reports, monitor and evaluate activities, audit expenditures and produce collect and analyze performance data. Applicants should provide CVs or resumes for key personnel along with a current organizational chart with their application.

Applicant must be able to manage program performance, evaluation, performance monitoring, financial reporting, and must have capacity to manage the required funds in accordance with the HHS Grants Policy Statement, which can be found at: http://www.hhs.gov/asfr/ogapa/aboutog/hhsgps107.pdf.

d. Work Plan

The work plan allows the CDC project officer to monitor implementation of activities and progress on period of performance outcomes. Each technical area the applicant is applying for must include a work plan. Work plans should be detailed and focused on the first year of the period of performance with a framework for subsequent years. Work plans should demonstrate alignment among the outcomes, strategies, activities, timelines and staffing. Applicants are encouraged to reference activities indicated under the "Strategy & Activities" section, but are

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encouraged to augment those activities on the basis of priority needs. Additional information on performance measures, data sources and target population(s) can also be included.

The work plan must include, at minimum:

- Activities and timelines to support achievement of expected outcomes. These activities must be in alignment with the overall logic model and have appropriate performance measures or milestones for accomplishing tasks;
- Work plans must include activities and performance measures that are specific, measurable, achievable, relevant and time-specific;
- Information on data sources and target population(s);
- Staff and administrative roles and functions to support implementation of the award; and
- Monitoring and evaluation plan to determine quality and effectiveness of program activities.

Within each project, applicant must submit separate Work Plans and Budgets. Applicant must clearly state how the proposed projects will impact public health and improve population health for the target population. Applicant must provide assurance of coordinated management and administrative support. Assessment of the ability of the program's principal investigators and program staff to lead a highly integrated program of the different program activities will be an important consideration in the evaluation of the application.

The core supports the administration and infrastructure costs of the program and should not be duplicated with any other components. The responsibilities and activities of the core include:

- Appropriate and adequate organization and facilities for activities such as computer support, workshops, seminars, and other activities that supports various activities.
- A data/statistical support activity may be included in the administrative core if needed for the center for various activities.

A sample work plan format is presented below to show how a traditional work plan aligns with the logic model and narrative. In this format, the table would be completed for each period of performance outcome. If a particular activity leads to multiple outcomes, it should be described under each outcome measure.

Period of performance Outcome: [from Outcomes section and/or logic model]		**Outcome Measure:** [from Evaluation and Performance Measurement section]	
Strategies and Activities	**Process Measure** [from Evaluation and Performance	**Responsible Position / Party**	**Completion Date**

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	Measurement section]		
1.			
2.			
3.			
4.			
5.			
6			

e. CDC Monitoring and Accountability Approach

Monitoring activities include routine and ongoing communication between CDC and recipients, site visits, and recipient reporting (including work plans, performance, and financial reporting). Consistent with applicable grants regulations and policies, CDC expects the following to be included in post-award monitoring for grants and cooperative agreements:

- Tracking recipient progress in achieving the desired outcomes.
- Ensuring the adequacy of recipient systems that underlie and generate data reports.
- Creating an environment that fosters integrity in program performance and results.

Monitoring may also include the following activities deemed necessary to monitor the award:

- Ensuring that work plans are feasible based on the budget and consistent with the intent of the award.
- Ensuring that recipients are performing at a sufficient level to achieve outcomes within stated timeframes.
- Working with recipients on adjusting the work plan based on achievement of outcomes, evaluation results and changing budgets.
- Monitoring performance measures (both programmatic and financial) to assure satisfactory performance levels.

Monitoring and reporting activities that assist grants management staff (e.g., grants management officers and specialists, and project officers) in the identification, notification, and management of high-risk recipients.

Monitoring activities include routine and ongoing communication between CDC and recipients, site visits, and recipient reporting (including work plans, performance, and financial reporting). Consistent with applicable grants regulations and policies, CDC expects the following to be included in post-award monitoring for grants and cooperative agreements:

- Tracking recipient progress in achieving the desired outcomes.

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- Ensuring the adequacy of recipient systems that underlie and generate data reports.

- Creating an environment that fosters integrity in program performance and results.

Monitoring may also include the following activities deemed necessary to monitor the award:

- Ensuring that work plans are feasible based on the budget and consistent with the intent of the award.

- Ensuring that recipients are performing at a sufficient level to achieve outcomes within stated timeframes.

- Working with recipients on adjusting the work plan based on achievement of outcomes, evaluation results and changing budgets.

- Monitoring performance measures (both programmatic and financial) to assure satisfactory performance levels.

Monitoring and reporting activities that assist grants management staff (e.g., grants management officers and specialists, and project officers) in the identification, notification, and management of high-risk grantees.

f. CDC Program Support to Recipients (THIS SECTION APPLIES ONLY TO COOPERATIVE AGREEMENTS)

In a cooperative agreement, CDC staff are substantially involved in the program activities, above and beyond routine grant monitoring. CDC activities for this program are as follows:

- Project management and technical oversight of project activities and on development and implementation of the evaluation performance management plan.
- Organize an orientation meeting with the recipient for a briefing on applicable U.S. Government, and HHS/CDC expectations, regulations and key management requirements, as well as report formats and contents.
- Meet on a regular basis with the recipient to assess expenditures in relation to approved work plan and modify plans as necessary.
- Review and approve the recipients' Evaluation and Performance Measurement Plan.
- Provide technical assistance, as mutually agreed upon, and revise annually during validation of the first and subsequent annual work plans.
- Assist the recipient in developing and implementing quality-assurance criteria and procedures.
- Facilitate in-country planning and review meetings for technical assistance activities.
- Coordinate activities where appropriate with other partners.
- Collaborate with the recipient on designing and implementing the activities listed above, including, but not limited to: the provision of technical assistance to develop program activities, data management and analysis and quality assurance.
- Facilitate in-country planning and review meetings for technical assistance activities.
- Provide in-country administrative support to help the recipient meet U.S. Government

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financial and reporting requirements approved by the Office of Management and Budget (OMB) under 0920-0428.

- Provide technical assistance or advice on any data collections on 10 or more people planned or conducted by the recipient. All such data collections-- where CDC staff will be or are approving, directing, conducting, managing, or owning data-- must undergo OMB project determinations by CDC and may require OMB PRA clearance prior to the start of the project.
- CDC must be included as co-investigators in any projects or publications resulting from awarded activities
- Provide technical oversight for all activities under this award.

B. Award Information

1. Funding Instrument Type:	Cooperative Agreement CDC's substantial involvement in this program appears in the CDC Program Support to Recipients Section.
2. Award Mechanism:	UE2

Emergency and Environmental Health Services

3. Fiscal Year:	2018
4. Approximate Total Fiscal Year Funding:	$5,000,000
5. Approximate Period of Performance Funding:	$25,000,000

This amount is subject to the availability of funds.

Estimated Total Funding:	$25,000,000
6. Approximate Period of Performance Length:	5 year(s)
7. Expected Number of Awards:	1
8. Approximate Average Award:	$5,000,000 Per Budget Period

This amount is subject to the availability of funds.

9. Award Ceiling:	$0 Per Budget Period

This amount is subject to the availability of funds.

There is no award ceiling for this NOFO, although the average award is $5M.

10. Award Floor:	$0 Per Budget Period
11. Estimated Award Date:	09/30/2018
12. Budget Period Length:	12 month(s)

Throughout the project period, CDC will continue the award based on the availability of funds, the evidence of satisfactory progress by the recipient (as documented in required reports), and the determination that continued funding is in the best interest of the federal government. The

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total number of years for which federal support has been approved (project period) will be shown in the "Notice of Award." This information does not constitute a commitment by the federal government to fund the entire period. The total period of performance comprises the initial competitive segment and any subsequent non-competitive continuation award(s).

13. Direct Assistance

Direct Assistance (DA) is not available through this FOA.

C. Eligibility Information

1. Eligible Applicants

Eligibility Category:	Others (see text field entitled "Additional Information on Eligibility" for clarification)
Additional Eligibility Category:	
Other:	Ministries of Health

2. Additional Information on Eligibility

Single eligibility

3. Justification for Less than Maximum Competition

Eligibility for funding under this NOFO is limited to the Chinese Center for Disease Control and Prevention (China CDC) in order to continue the partnership between the National Public Health Institutes of the US and China --U.S. CDC and China CDC. China CDC is the only appropriate and qualified agency to conduct the activities specified under this NOFO for the following reasons:

- There is one China CDC which provides essential public health services for the population of China. China CDC is the only organization which has the legal mandate and obligation and technical capacity to implement activities supported in this NOFO.
- The MOU that established the mechanism and authority for U.S. CDC's presence in China and to carry out public health programs of mutual interest to the U.S. and China requires that U.S. CDC work directly with China CDC. Moreover, U.S. CDC's presence in China is at the invitation of the Chinese government to collaborate with its sanctioned public health agencies, i.e., China CDC, and not to other outside entities.
- China CDC is the key governmental agency that will enable U.S. CDC to execute its approved global health strategy. Specifically, U.S. CDC working through its strong

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partnerships with China CDC, will assure the implementation and evaluation of public health programs that will result in the prevention and control of infectious diseases in China that have global impact.

- China CDC is the only organization in China that has the requisite scientific and technical expertise, the infrastructure capacity, and legal mandate and obligation from the Chinese government to provide technical advice and execute essential public health functions and innovative disease control approaches as outlined in this cooperative agreement. It also has the expertise and mandate within the government of China to provide technical assistance to other countries. These combined attributes make China CDC uniquely qualified as the only organization in China capable of effectively conducting the activities proposed for this NOFO.

China CDC has been a technically capable and fiscally responsible partner in prior cooperative agreements with CDC over the past 16 years. With catalytic US CDC funding to provide critical technical guidance and support innovative pilot projects, China CDC has been able to more effectively invest its own public health funds to strengthen its programs in field epidemiology training, influenza surveillance and control, emerging infections, TB surveillance, NCD, HIV/AIDS prevention, care, training, and surveillance, and to scale up successful pilot projects. As a result of this success and in light of China's increasing capacity, improved health status, and expanding global role, the nature of the collaboration is evolving from one of assistance to one of partnership. This cooperative agreement with China CDC will build on this successful partnership by providing specialized technical support to China to continue developing its public health capabilities to reduce the threat of infectious diseases while also contributing to improving public health globally.

4. Cost Sharing or Matching

Cost Sharing / Matching Requirement: No

5. Maintenance of Effort

Maintenance of effort is not required for this program.

D. Application and Submission Information

1. Required Registrations

An organization must be registered at the three following locations before it can submit an application for funding at www.grants.gov.

a. Data Universal Numbering System:

All applicant organizations must obtain a Data Universal Numbering System (DUNS) number. A DUNS number is a unique nine-digit identification number provided by Dun & Bradstreet (D&B). It will be used as the Universal Identifier when applying for federal awards or cooperative agreements.

The applicant organization may request a DUNS number by telephone at 1-866-705-5711 (toll free) or internet at http:// fedgov.dnb. com/ webform/ displayHomePage.do. The DUNS number will be provided at no charge.

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If funds are awarded to an applicant organization that includes sub-recipients, those sub-recipients must provide their DUNS numbers before accepting any funds.

b. System for Award Management (SAM):
The SAM is the primary registrant database for the federal government and the repository into which an entity must submit information required to conduct business as a recipient. All applicant organizations must register with SAM, and will be assigned a SAM number. All information relevant to the SAM number must be current at all times during which the applicant has an application under consideration for funding by CDC. If an award is made, the SAM information must be maintained until a final financial report is submitted or the final payment is received, whichever is later. The SAM registration process can require 10 or more business days, and registration must be renewed annually. Additional information about registration procedures may be found at ***www.SAM.gov***.

c. Grants.gov:
The first step in submitting an application online is registering your organization at www.grants.gov, the official HHS E-grant Web site. Registration information is located at the "Get Registered" option at www.grants.gov.
All applicant organizations must register at www.grants.gov. The one-time registration process usually takes not more than five days to complete. Applicants should start the registration process as early as possible.

Step	System	Requirements	Duration	Follow Up
1	Data Universal Number System (DUNS)	1. Click on http://fedgov.dnb.com/webform 2. Select Begin DUNS search/request process 3. Select your country or territory and follow the instructions to obtain your DUNS 9-digit # 4. Request appropriate staff member(s) to obtain DUNS number, verify & update information under DUNS number	1-2 Business Days	To confirm that you have been issued a new DUNS number check online at (http://fedgov.dnb.com/webform) or call 1-866-705-5711
2	System for Award Management (SAM) formerly Central Contractor Registration (CCR)	1. Retrieve organizations DUNS number 2. Go to www.sam.gov	3-5 Business Days but up to 2 weeks and designate an E-Biz	For SAM Customer Service Contact POC (note CCR username will not work in SAM and you will need to have an

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active SAM account before you can register on	and must be renewed once a year	https://fsd.gov/fsd-gov/home.do Calls: 866-606-822	0

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		grants.gov)		
3	Grants.gov	1. Set up an individual account in Grants.gov using organization new DUNS number to become an authorized organization representative (AOR) 2. Once the account is set up the E-BIZ POC will be notified via email 3. Log into grants.gov using the password the E-BIZ POC received and create new password 4. This authorizes the AOR to submit applications on behalf of the organization	Same day but can take 8 weeks to be fully registered and approved in the system (note, applicants MUST obtain a DUNS number and SAM account before applying on grants.gov)	Register early! Log into grants.gov and check AOR status until it shows you have been approved

2. Request Application Package

Applicants may access the application package at www.grants.gov.

3. Application Package

Applicants must download the SF-424, Application for Federal Assistance, package associated with this notice of funding opportunity at www.grants.gov. If Internet access is not available, or if the online forms cannot be accessed, applicants may call the CDC OGS staff at 770-488-2700 or e-mail OGS ogstims@cdc.gov for assistance. Persons with hearing loss may access CDC telecommunications at TTY 1-888-232-6348.

4. Submission Dates and Times

If the application is not submitted by the deadline published in the NOFO, it will not be processed. Office of Grants Services (OGS) personnel will notify the applicant that their application did not meet the deadline. The applicant must receive pre-approval to submit a paper application (see Other Submission Requirements section for additional details). If the applicant is authorized to submit a paper application, it must be received by the deadline provided by OGS.

a. Letter of Intent Deadline (must be emailed or postmarked by)

Due Date for Letter of Intent: **N/A**

b. Application Deadline

Due Date for Applications: **06/15/2018** , 11:59 p.m. U.S. Eastern Standard Time, at www.grants.gov. If Grants.gov is inoperable and cannot receive applications, and circumstances preclude advance notification of an extension, then applications must be submitted by the first

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business day on which grants.gov operations resume.

Date for Information Conference Call
N/A

5. CDC Assurances and Certifications

All applicants are required to sign and submit "Assurances and Certifications" documents indicated at http://wwwn.cdc.gov/ grantassurances/ (S(mj444mxct51lnrv1hljjjmaa)) /Homepage.aspx.
Applicants may follow either of the following processes:

- Complete the applicable assurances and certifications with each application submission, name the file "Assurances and Certifications" and upload it as a PDF file with at www.grants.gov
- Complete the applicable assurances and certifications and submit them directly to CDC on an annual basis at http://wwwn.cdc.gov/ grantassurances/ (S(mj444mxct51lnrv1hljjjmaa))/ Homepage.aspx

Assurances and certifications submitted directly to CDC will be kept on file for one year and will apply to all applications submitted to CDC by the applicant within one year of the submission date.

Duplication of Efforts
Applicants are responsible for reporting if this application will result in programmatic, budgetary, or commitment overlap with another application or award (i.e. grant, cooperative agreement, or contract) submitted to another funding source in the same fiscal year. Programmatic overlap occurs when (1) substantially the same project is proposed in more than one application or is submitted to two or more funding sources for review and funding consideration or (2) a specific objective and the project design for accomplishing the objective are the same or closely related in two or more applications or awards, regardless of the funding source. Budgetary overlap occurs when duplicate or equivalent budgetary items (e.g., equipment, salaries) are requested in an application but already are provided by another source. Commitment overlap occurs when an individual's time commitment exceeds 100 percent, whether or not salary support is requested in the application. Overlap, whether programmatic, budgetary, or commitment of an individual's effort greater than 100 percent, is not permitted. Any overlap will be resolved by the CDC with the applicant and the PD/PI prior to award. Report Submission: The applicant must upload the report in Grants.gov under "Other Attachment Forms." The document should be labeled: "Report on Programmatic, Budgetary, and Commitment Overlap."

6. Content and Form of Application Submission

Applicants are required to include all of the following documents with their application package at www.grants.gov.

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7. Letter of Intent

N/A

8. Table of Contents

(There is no page limit. The table of contents is not included in the project narrative page limit.): The applicant must provide, as a separate attachment, the "Table of Contents" for the entire submission package.
Provide a detailed table of contents for the entire submission package that includes all of the documents in the application and headings in the "Project Narrative" section. Name the file "Table of Contents" and upload it as a PDF file under "Other Attachment Forms" at www.grants.gov.

9. Project Abstract Summary

(Maximum 1 page)
A project abstract is included on the mandatory documents list and must be submitted at www.grants.gov. The project abstract must be a self-contained, brief summary of the proposed project including the purpose and outcomes. This summary must not include any proprietary or confidential information. Applicants must enter the summary in the "Project Abstract Summary" text box at www.grants.gov.

10. Project Narrative

(Unless specified in the "H. Other Information" section, maximum of 20 pages, single spaced, 12 point font, 1-inch margins, number all pages. This includes the work plan. Content beyond the specified page number will not be reviewed.)
Applicants must submit a Project Narrative with the application forms. Applicants must name this file "Project Narrative" and upload it at www.grants.gov. The Project Narrative must include **all** of the following headings (including subheadings): Background, Approach, Applicant Evaluation and Performance Measurement Plan, Organizational Capacity of Applicants to Implement the Approach, and Work Plan. The Project Narrative must be succinct, self-explanatory, and in the order outlined in this section. It must address outcomes and activities to be conducted over the entire period of performance as identified in the CDC Project Description section. Applicants should use the federal plain language guidelines and Clear Communication Index to respond to this Notice of Funding Opportunity. Note that recipients should also use these tools when creating public communication materials supported by this NOFO. Failure to follow the guidance and format may negatively impact scoring of the application.

a. Background

Applicants must provide a description of relevant background information that includes the context of the problem (See CDC Background).

b. Approach

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i. Purpose
Applicants must describe in 2-3 sentences specifically how their application will address the public health problem as described in the CDC Background section.

ii. Outcomes
Applicants must clearly identify the outcomes they expect to achieve by the end of the project period, as identified in the logic model in the Approach section of the CDC Project Description. Outcomes are the results that the program intends to achieve and usually indicate the intended direction of change (e.g., increase, decrease).

iii. Strategies and Activities
Applicants must provide a clear and concise description of the strategies and activities they will use to achieve the period of performance outcomes. Applicants must select existing evidence-based strategies that meet their needs, or describe in the Applicant Evaluation and Performance Measurement Plan how these strategies will be evaluated over the course of the project period. See the Strategies and Activities section of the CDC Project Description.

1. Collaborations
Applicants must describe how they will collaborate with programs and organizations either internal or external to CDC. Applicants must address the Collaboration requirements as described in the CDC Project Description.

2. Target Populations and Health Disparities
Applicants must describe the specific target population(s) in their jurisdiction and explain how such a target will achieve the goals of the award and/or alleviate health disparities. The applicants must also address how they will include specific populations that can benefit from the program that is described in the Approach section. Applicants must address the Target Populations and Health Disparities requirements as described in the CDC Project Description.

c. Applicant Evaluation and Performance Measurement Plan
Applicants must provide an evaluation and performance measurement plan that demonstrates how the recipient will fulfill the requirements described in the CDC Evaluation and Performance Measurement and Project Description sections of this NOFO. At a minimum, the plan must describe:

- How applicant will collect the performance measures, respond to the evaluation questions, and use evaluation findings for continuous program quality improvement. The Paperwork Reduction Act of 1995 (PRA): Applicants are advised that any activities involving information collections (e.g., surveys, questionnaires, applications, audits, data requests, reporting, recordkeeping and disclosure requirements) from 10 or more individuals or non-Federal entities, including State and local governmental agencies, and funded or sponsored by the Federal Government are subject to review and approval by the Office of Management and Budget. For further information about CDC's

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requirements under PRA see http://www.hhs.gov/ ocio/policy/collection/.

- How key program partners will participate in the evaluation and performance measurement planning processes.
- Available data sources, feasibility of collecting appropriate evaluation and performance data, data management plan (DMP), and other relevant data information (e.g., performance measures proposed by the applicant).

Where the applicant chooses to, or is expected to, take on specific evaluation studies, they should be directed to:

- Describe the type of evaluations (i.e., process, outcome, or both).
- Describe key evaluation questions to be addressed by these evaluations.
- Describe other information (e.g., measures, data sources).

Recipients will be required to submit a more detailed Evaluation and Performance Measurement plan (including the DMP elements) within the first 6 months of award, as described in the Reporting Section of this NOFO.

d. Organizational Capacity of Applicants to Implement the Approach

Applicants must address the organizational capacity requirements as described in the CDC Project Description.

11. Work Plan

(Included in the Project Narrative's page limit)
Applicants must prepare a work plan consistent with the CDC Project Description Work Plan section. The work plan integrates and delineates more specifically how the recipient plans to carry out achieving the period of performance outcomes, strategies and activities, evaluation and performance measurement.

12. Budget Narrative

Applicants must submit an itemized budget narrative. When developing the budget narrative, applicants must consider whether the proposed budget is reasonable and consistent with the purpose, outcomes, and program strategy outlined in the project narrative. The budget must include:

- Salaries and wages
- Fringe benefits
- Consultant costs
- Equipment
- Supplies
- Travel

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- Other categories
- Contractual costs
- Total Direct costs
- Total Indirect costs

Indirect costs could include the cost of collecting, managing, sharing and preserving data. Indirect costs on grants awarded to foreign organizations and foreign public entities and performed fully outside of the territorial limits of the U.S. may be paid to support the costs of compliance with federal requirements at a fixed rate of eight percent of MTDC exclusive of tuition and related fees, direct expenditures for equipment, and subawards in excess of $25,000. Negotiated indirect costs may be paid to the American University, Beirut, and the World Health Organization.

If applicable and consistent with the cited statutory authority for this announcement, applicant entities may use funds for activities as they relate to the intent of this NOFO to meet national standards or seek health department accreditation through the Public Health Accreditation Board (see: http://www.phaboard.org). Applicant entities to whom this provision applies include state, local, territorial governments (including the District of Columbia, the Commonwealth of Puerto Rico, the Virgin Islands, the Commonwealth of the Northern Marianna Islands, American Samoa, Guam, the Federated States of Micronesia, the Republic of the Marshall Islands, and the Republic of Palau), or their bona fide agents, political subdivisions of states (in consultation with states), federally recognized or state-recognized American Indian or Alaska Native tribal governments, and American Indian or Alaska Native tribally designated organizations. Activities include those that enable a public health organization to deliver public health services such as activities that ensure a capable and qualified workforce, up-to-date information systems, and the capability to assess and respond to public health needs. Use of these funds must focus on achieving a minimum of one national standard that supports the intent of the NOFO. Proposed activities must be included in the budget narrative and must indicate which standards will be addressed.

Applicants must name this file "Budget Narrative" and upload it as a PDF file at www.grants.gov. If requesting indirect costs in the budget, a copy of the indirect cost-rate agreement is required. If the indirect costs are requested, include a copy of the current negotiated federal indirect cost rate agreement or a cost allocation plan approval letter for those Recipients under such a plan. Applicants must name this file "Indirect Cost Rate" and upload it at www.grants.gov.

13. Funds Tracking

Proper fiscal oversight is critical to maintaining public trust in the stewardship of federal funds. Effective October 1, 2013, a new HHS policy on subaccounts requires the CDC to set up payment subaccounts within the Payment Management System (PMS) for all new grant awards. Funds awarded in support of approved activities and drawdown instructions will be identified on the Notice of Award in a newly established PMS subaccount (P subaccount). Recipients will be required to draw down funds from award-specific accounts in the PMS. Ultimately, the subaccounts will provide recipients and CDC a more detailed and precise understanding of financial transactions. The successful applicant will be required to track funds by P-accounts/sub accounts for each project/cooperative agreement awarded. Applicants are

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encouraged to demonstrate a record of fiscal responsibility and the ability to provide sufficient and effective oversight. Financial management systems must meet the requirements as described 2 CFR 200 which include, but are not limited to, the following:

- Records that identify adequately the source and application of funds for federally-funded activities.
- Effective control over, and accountability for, all funds, property, and other assets.
- Comparison of expenditures with budget amounts for each Federal award.
- Written procedures to implement payment requirements.
- Written procedures for determining cost allowability.
- Written procedures for financial reporting and monitoring.

14. Intergovernmental Review

Executive Order 12372 does not apply to this program.

15. Pilot Program for Enhancement of Employee Whistleblower Protections

Pilot Program for Enhancement of Employee Whistleblower Protections: All applicants will be subject to a term and condition that applies the terms of 48 Code of Federal Regulations (CFR) section 3.908 to the award and requires that recipients inform their employees in writing (in the predominant native language of the workforce) of employee whistleblower rights and protections under 41 U.S.C. 4712.

16. Copyright Interests Provisions

This provision is intended to ensure that the public has access to the results and accomplishments of public health activities funded by CDC. Pursuant to applicable grant regulations and CDC's Public Access Policy, Recipient agrees to submit into the National Institutes of Health (NIH) Manuscript Submission (NIHMS) system an electronic version of the final, peer-reviewed manuscript of any such work developed under this award upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. Also at the time of submission, Recipient and/or the Recipient's submitting author must specify the date the final manuscript will be publicly accessible through PubMed Central (PMC). Recipient and/or Recipient's submitting author must also post the manuscript through PMC within twelve (12) months of the publisher's official date of final publication; however the author is strongly encouraged to make the subject manuscript available as soon as possible. The recipient must obtain prior approval from the CDC for any exception to this provision.

The author's final, peer-reviewed manuscript is defined as the final version accepted for journal publication, and includes all modifications from the publishing peer review process, and all graphics and supplemental material associated with the article. Recipient and its submitting authors working under this award are responsible for ensuring that any publishing or copyright agreements concerning submitted articles reserve adequate right to fully comply with this provision and the license reserved by CDC. The manuscript will be hosted in both PMC and the

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CDC Stacks institutional repository system. In progress reports for this award, recipient must identify publications subject to the CDC Public Access Policy by using the applicable NIHMS identification number for up to three (3) months after the publication date and the PubMed Central identification number (PMCID) thereafter.

17. Funding Restrictions

Restrictions that must be considered while planning the programs and writing the budget are:

- Recipients may not use funds for research.
- Recipients may not use funds for clinical care except as allowed by law.
- Recipients may use funds only for reasonable program purposes, including personnel, travel, supplies, and services.
- Generally, recipients may not use funds to purchase furniture or equipment. Any such proposed spending must be clearly identified in the budget.
- Reimbursement of pre-award costs generally is not allowed, unless the CDC provides written approval to the recipient.
- Other than for normal and recognized executive-legislative relationships, no funds may be used for:
    - publicity or propaganda purposes, for the preparation, distribution, or use of any material designed to support or defeat the enactment of legislation before any legislative body
    - the salary or expenses of any grant or contract recipient, or agent acting for such recipient, related to any activity designed to influence the enactment of legislation, appropriations, regulation, administrative action, or Executive order proposed or pending before any legislative body
- See Additional Requirement (AR) 12 for detailed guidance on this prohibition and additional guidance on lobbying for CDC recipients.
- The direct and primary recipient in a cooperative agreement program must perform a substantial role in carrying out project outcomes and not merely serve as a conduit for an award to another party or provider who is ineligible.
- In accordance with the United States Protecting Life in Global Health Assistance policy, all non-governmental organization (NGO) applicants acknowledge that foreign NGOs that receive funds provided through this award, either as a prime recipient or subrecipient, are strictly prohibited, regardless of the source of funds, from performing abortions as a method of family planning or engaging in any activity that promotes abortion as a method of family planning, or to provide financial support to any other foreign non-governmental organization that conducts such activities. See Additional Requirement (AR) 35 for applicability (https://www.cdc.gov/grants/additionalrequirements/ar-35.html).

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18. Data Management Plan

As identified in the Evaluation and Performance Measurement section, applications involving data collection must include a Data Management Plan (DMP) as part of their evaluation and performance measurement plan. The DMP is the applicant's assurance of the quality of the public health data through the data's lifecycle and plans to deposit data in a repository to preserve and to make the data accessible in a timely manner. See web link for additional information:
https://www.cdc.gov/grants/additionalrequirements/ar-25.html

19. Other Submission Requirements

a. Electronic Submission: Applications must be submitted electronically at www.grants.gov. The application package can be downloaded at www.grants.gov. Applicants can complete the application package off-line and submit the application by uploading it at www.grants.gov. All application attachments must be submitted using a PDF file format. Directions for creating PDF files can be found at www.grants.gov. File formats other than PDF may not be readable by OGS Technical Information Management Section (TIMS) staff.

Applications must be submitted electronically by using the forms and instructions posted for this notice of funding opportunity at www.grants.gov.

If Internet access is not available or if the forms cannot be accessed online, applicants may contact the OGS TIMS staff at 770-488-2700 or by e-mail at ogstims@cdc.gov, Monday through Friday, 7:30 a.m.–4:30 p.m., except federal holidays. Electronic applications will be considered successful if they are available to OGS TIMS staff for processing from www.grants.gov on the deadline date.

b. Tracking Number: Applications submitted through www.grants.gov are time/date stamped electronically and assigned a tracking number. The applicant's Authorized Organization Representative (AOR) will be sent an e-mail notice of receipt when www.grants.gov receives the application. The tracking number documents that the application has been submitted and initiates the required electronic validation process before the application is made available to CDC.

c. Validation Process: Application submission is not concluded until the validation process is completed successfully. After the application package is submitted, the applicant will receive a "submission receipt" e-mail generated by www.grants.gov. A second e-mail message to applicants will then be generated by www.grants.gov that will either validate or reject the submitted application package. This validation process may take as long as two business days. Applicants are strongly encouraged to check the status of their application to ensure that submission of their package has been completed and no submission errors have occurred. Applicants also are strongly encouraged to allocate ample time for filing to guarantee that their application can be submitted and validated by the deadline published in the NOFO. Non-validated applications will not be accepted after the published application deadline date.

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If you do not receive a "validation" e-mail within two business days of application submission, please contact www.grants.gov. For instructions on how to track your application, refer to the e-mail message generated at the time of application submission or the Grants.gov Online User Guide.
https:// www.grants.gov/help/html/help/index.htm? callingApp=custom#t= Get_Started%2FGet_Started. htm

d. Technical Difficulties: If technical difficulties are encountered at www.grants.gov, applicants should contact Customer Service at www.grants.gov. The www.grants.gov Contact Center is available 24 hours a day, 7 days a week, except federal holidays. The Contact Center is available by phone at 1-800-518-4726 or by e-mail at support@grants.gov. Application submissions sent by e-mail or fax, or on CDs or thumb drives will not be accepted. Please note that www.grants.gov is managed by HHS.

e. Paper Submission: If technical difficulties are encountered at www.grants.gov, applicants should call the www.grants.gov Contact Center at 1-800-518-4726 or e-mail them at support@grants.gov for assistance. After consulting with the Contact Center, if the technical difficulties remain unresolved and electronic submission is not possible, applicants may e-mail CDC GMO/GMS, before the deadline, and request permission to submit a paper application. Such requests are handled on a case-by-case basis.
An applicant's request for permission to submit a paper application must:

1. Include the www.grants.gov case number assigned to the inquiry
2. Describe the difficulties that prevent electronic submission and the efforts taken with the www.grants.gov Contact Center to submit electronically; and
3. Be received via e-mail to the GMS/GMO listed below at least three calendar days before the application deadline. Paper applications submitted without prior approval will not be considered.

   If a paper application is authorized, OGS will advise the applicant of specific instructions for submitting the application (e.g., original and two hard copies of the application by U.S. mail or express delivery service).

E. Review and Selection Process

1. Review and Selection Process: Applications will be reviewed in three phases

a. Phase 1 Review
All applications will be initially reviewed for eligibility and completeness by CDC Office of Grants Services. Complete applications will be reviewed for responsiveness by the Grants Management Officials and Program Officials. Non-responsive applications will not advance to Phase II review. Applicants will be notified that their applications did not meet eligibility and/or published submission requirements.

b. Phase II Review

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A review panel will evaluate complete, eligible applications in accordance with the criteria below.
i. Approach
ii. Evaluation and Performance Measurement
iii. Applicant's Organizational Capacity to Implement the Approach
Not more than thirty days after the Phase II review is completed, applicants will be notified electronically if their application does not meet eligibility or published submission requirements.

i. Approach	Maximum Points:40

Evaluate the extent to which the applicant:

- Includes an overall strategy, including measurable timelines, clear monitoring and evaluation procedures, and specific activities for meeting the proposed outcomes. Is this strategy consistent with CDC priorities as defined in the purpose section of this NOFO? ((1) Increase the global availability of high quality scientific information for public health decision making and response; (2) Translate scientific information into evidence-based policy and disease prevention and control programs; (3) Increase global public health capacity in China, Asia, and Africa; and (4) Develop and reinforce sustainable global health collaboration between the US and China.);
- Describes activities that are evidence-based, realistic, achievable, measurable and culturally appropriate in the Chinese context to achieve the goals of the NOFO;
- Shows that the proposed use of funds is an efficient and effective way to implement the strategies and activities and attain the period of performance outcomes;
- Includes reasonable estimates of activity outputs (i.e., the numbers of sites to be supported by a proposed sentinel disease surveillance program, number of patients the surveillance program will reach, etc.);
- Demonstrates a clear and concise understanding of the current public health problem and context relevant to the programmatic areas targeted;
- Identifies relevant partnerships with other local Chinese and international organizations that will be important for implementation success;
- Presents a work plan that is aligned with the strategies/activities, outcomes, and performance measures in the approach and is consistent with the content and format proposed by CDC.

ii. Evaluation and Performance Measurement	Maximum Points:30

Evaluate the extent to which the applicant:

- Shows/affirms the ability to collect data on the process and outcome performance measures specified by CDC in the project description and presented by the applicant in their approach;
- Describes clear monitoring and evaluation procedures and how evaluation and performance measurement will be incorporated into planning, implementation, and reporting of project activities;
- Describes how performance measurement and evaluation findings will be reported, and

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used to demonstrate the outcomes of the NOFO and for continuous program quality improvement;

- Shows plan for dissemination of evaluation findings, including for national and international forums, in order to enhance availability and quality of public health information in China;
- Describes how evaluation and performance measurement will contribute to developing an evidence base for programs that lack a strong effectiveness evidence base, and describes how data collected will contribute to local and national level policy setting for improved disease detection and control within and outside China;
- Describes any evaluation studies the recipient will undertake. Describe in sufficient detail to identify the key evaluation questions, and data sources and analysis methods.
- Includes a preliminary Data Management Plan (DMP), if applicable.

iii. Applicant's Organizational Capacity to Implement the Approach	Maximum Points:30

Evaluate the extent to which the applicant addresses the items below.

- Demonstrates relevant experience and capacity (management, administrative, and technical) to implement the activities and achieve the project outcomes. Applicant should demonstrate ability to both manage project at national level and at the local implementation level in China;
- Demonstrates experience and capacity to implement the evaluation plan, including demonstrating staffing capacity to conduct monitoring and evaluation activities;
- Provides a staffing plan and project management structure that will be sufficient to achieve the project outcomes and which clearly defines staff roles with regard to implementation and management of projects. Provides an organizational chart, CVs and resumes, etc.;
- Provides a clear plan for the administration and management of the proposed activities, and to manage the resources of the program, prepare reports, monitor and evaluate activities, audit expenditures and produce, collect, and analyze performance data.
- Demonstrates technical expertise in public health practice.

Budget

When scoring budgets, CDC programs will assess whether the budget aligns with the proposed work plans. For additional guidance, check with the Center for Global Health Extramural Program Office, GMO or GMS.

Not more than thirty days after the Phase II review is completed, applicants will be notified electronically if their application does not meet eligibility or published submission requirements.

c. Phase III Review

N/A for this sole source award.

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Review of risk posed by applicants.
Prior to making a Federal award, CDC is required by 31 U.S.C. 3321 and 41 U.S.C. 2313 to review information available through any OMB-designated repositories of government-wide eligibility qualification or financial integrity information as appropriate. See also suspension and debarment requirements at 2 CFR parts 180 and 376.
In accordance 41 U.S.C. 2313, CDC is required to review the non-public segment of the OMB-designated integrity and performance system accessible through SAM (currently the Federal Recipient Performance and Integrity Information System (FAPIIS)) prior to making a Federal award where the Federal share is expected to exceed the simplified acquisition threshold, defined in 41 U.S.C. 134, over the period of performance. At a minimum, the information in the system for a prior Federal award recipient must demonstrate a satisfactory record of executing programs or activities under Federal grants, cooperative agreements, or procurement awards; and integrity and business ethics. CDC may make a Federal award to a recipient who does not fully meet these standards, if it is determined that the information is not relevant to the current Federal award under consideration or there are specific conditions that can appropriately mitigate the effects of the non-Federal entity's risk in accordance with 45 CFR §75.207.
CDC's framework for evaluating the risks posed by an applicant may incorporate results of the evaluation of the applicant's eligibility or the quality of its application. If it is determined that a Federal award will be made, special conditions that correspond to the degree of risk assessed may be applied to the Federal award. The evaluation criteria is described in this Notice of Funding Opportunity.
In evaluating risks posed by applicants, CDC will use a risk-based approach and may consider any items such as the following:
(1) Financial stability;
(2) Quality of management systems and ability to meet the management standards prescribed in this part;
(3) History of performance. The applicant's record in managing Federal awards, if it is a prior recipient of Federal awards, including timeliness of compliance with applicable reporting requirements, conformance to the terms and conditions of previous Federal awards, and if applicable, the extent to which any previously awarded amounts will be expended prior to future awards;
(4) Reports and findings from audits performed under subpart F 45 CFR 75 or the reports and findings of any other available audits; and
(5) The applicant's ability to effectively implement statutory, regulatory, or other requirements imposed on non-Federal entities.
CDC must comply with the guidelines on government-wide suspension and debarment in 2 CFR part 180, and require non-Federal entities to comply with these provisions. These provisions restrict Federal awards, subawards and contracts with certain parties that are debarred, suspended or otherwise excluded from or ineligible for participation in Federal programs or activities.

2. Announcement and Anticipated Award Dates

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F. Award Administration Information

1. Award Notices

Recipients will receive an electronic copy of the Notice of Award (NOA) from CDC OGS. The NOA shall be the only binding, authorizing document between the recipient and CDC. The NOA will be signed by an authorized GMO and emailed to the Recipient Business Officer listed in application and the Program Director.

Any applicant awarded funds in response to this Notice of Funding Opportunity will be subject to the DUNS, SAM Registration, and Federal Funding Accountability And Transparency Act Of 2006 (FFATA) requirements.

Unsuccessful applicants will receive notification of these results by e-mail with delivery receipt or by U.S. mail.

The recipient will receive an electronic copy of their NOFO from CDC OGS.

2. Administrative and National Policy Requirements

Recipients must comply with the administrative and public policy requirements outlined in 45 CFR Part 75 and the HHS Grants Policy Statement, as appropriate.
Brief descriptions of relevant provisions are available at http://www.cdc.gov/grants/additionalrequirements/index.html#ui-id-17.
The HHS Grants Policy Statement is available at http://www.hhs.gov/sites/default/files/grants/grants/policies-regulations/hhsgps107.pdf.

For more information on the CFR visit http://www. access.gpo.gov/ nara/cfr/cfr-table-search.html

3. Reporting

Reporting provides continuous program monitoring and identifies successes and challenges that recipients encounter throughout the project period. Also, reporting is a requirement for recipients who want to apply for yearly continuation of funding. Reporting helps CDC and recipients because it:

- Helps target support to recipients;
- Provides CDC with periodic data to monitor recipient progress toward meeting the Notice of Funding Opportunity outcomes and overall performance;
- Allows CDC to track performance measures and evaluation findings for continuous quality and program improvement throughout the period of performance and to determine applicability of evidence-based approaches to different populations, settings, and contexts; and

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- Enables CDC to assess the overall effectiveness and influence of the NOFO.

The table below summarizes required and optional reports. All required reports must be sent electronically to GMS listed in the "Agency Contacts" section of the NOFO copying the CDC Project Officer.

Report	When?	Required?
Recipient Evaluation and Performance Measurement Plan, including Data Management Plan (DMP)	6 months into award	Yes
Annual Performance Report (APR)	No later than 120 days before end of budget period. Serves as yearly continuation application.	Yes
Federal Financial Reporting Forms	90 days after end of calendar quarter in which budget period ends	Yes
Final Performance and Financial Report	90 days after end of performance period.	Yes
Payment Management System (PMS) Reporting	Quarterly reports due January 30; April 30, July 30, and October 30 each year.	Yes

a. Recipient Evaluation and Performance Measurement Plan (required)
With support from CDC, recipients must elaborate on their initial applicant evaluation and performance measurement plan. This plan must be no more than 20 pages; recipients must submit the plan 6 months into the award. HHS/CDC will review and approve the recipient's monitoring and evaluation plan to ensure that it is appropriate for the activities to be undertaken as part of the agreement, for compliance with the monitoring and evaluation guidance established by HHS/CDC, or other guidance otherwise applicable to this Agreement.

Recipient Evaluation and Performance Measurement Plan (required): This plan should provide additional detail on the following:

Performance Measurement

- Performance measures and targets
- The frequency that performance data are to be collected.
- How performance data will be reported.

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• How quality of performance data will be assured.
• How performance measurement will yield findings to demonstrate progress towards achieving NOFO goals (e.g., reaching target populations or achieving expected outcomes).
• Dissemination channels and audiences.
• Other information requested as determined by the CDC program.

Evaluation

• The types of evaluations to be conducted (e.g. process or outcome evaluations).
• The frequency that evaluations will be conducted.
• How evaluation reports will be published on a publically available website.
• How evaluation findings will be used to ensure continuous quality and program improvement.
• How evaluation will yield findings to demonstrate the value of the NOFO (e.g., effect on improving public health outcomes, effectiveness of NOFO, cost-effectiveness or cost-benefit).
• Dissemination channels and audiences.

HHS/CDC or its designee will also undertake monitoring and evaluation of the defined activities within the agreement. The recipient must ensure reasonable access by HHS/CDC or its designee to all necessary sites, documentation, individuals and information to monitor, evaluate and verify the appropriate implementation the activities and use of HHS/CDC funding under this Agreement.

b. Annual Performance Report (APR) (required)

The recipient must submit the APR via www.Grantsolutions.gov no later than120 days prior to the end of the budget period. This report must not exceed 45 pages excluding administrative reporting. Attachments are not allowed, but web links are allowed.
This report must include the following:

- **Performance Measures:** Recipients must report on performance measures for each budget period and update measures, if needed.
- **Evaluation Results:** Recipients must report evaluation results for the work completed to date (including findings from process or outcome evaluations).
- **Work Plan:** Recipients must update work plan each budget period to reflect any changes in period of performance outcomes, activities, timeline, etc.
- **Successes**
    - Recipients must report progress on completing activities and progress towards achieving the period of performance outcomes described in the logic model and work plan.
    - Recipients must describe any additional successes (e.g. identified through evaluation results or lessons learned) achieved in the past year.
    - Recipients must describe success stories.
- **Challenges**
    - Recipients must describe any challenges that hindered or might hinder their ability to complete the work plan activities and achieve the period of

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performance outcomes.
    - Recipients must describe any additional challenges (e.g., identified through evaluation results or lessons learned) encountered in the past year.
- **CDC Program Support to Recipients**
    - Recipients must describe how CDC could help them overcome challenges to complete activities in the work plan and achieving period of performance outcomes.
- **Administrative Reporting** (No page limit)
    - SF-424A Budget Information-Non-Construction Programs.
    - Budget Narrative – Must use the format outlined in "Content and Form of Application Submission, Budget Narrative" section.
    - Indirect Cost Rate Agreement.

The recipients must submit the Annual Performance Report via www.Grantsolutions.gov no later than 120 days prior to the end of the budget period.

c. Performance Measure Reporting (optional)
CDC programs may require more frequent reporting of performance measures than annually in the APR. If this is the case, CDC programs must specify reporting frequency, data fields, and format for recipients at the beginning of the award period.

N/A

d. Federal Financial Reporting (FFR) (required)
The annual FFR form (SF-425) is required and must be submitted 90 days after the end of the budget period. The report must include only those funds authorized and disbursed during the timeframe covered by the report. The final FFR must indicate the exact balance of unobligated funds, and may not reflect any unliquidated obligations. There must be no discrepancies between the final FFR expenditure data and the Payment Management System's (PMS) cash transaction data. Failure to submit the required information by the due date may adversely affect the future funding of the project. If the information cannot be provided by the due date, recipients are required to submit a letter of explanation to OGS and include the date by which the Grants Officer will receive information.

e. Final Performance and Financial Report (required)
This report is due 90 days after the end of the period of performance. CDC programs must indicate that this report should not exceed 40 pages. This report covers the entire period of performance and can include information previously reported in APRs. At a minimum, this report must include the following:

- Performance Measures – Recipients must report final performance data for all process and outcome performance measures.
- Evaluation Results – Recipients must report final evaluation results for the period of performance for any evaluations conducted.

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- Impact/Results/Success Stories – Recipients must use their performance measure results and their evaluation findings to describe the effects or results of the work completed over the project period, and can include some success stories.
- A final Data Management Plan that includes the location of the data collected during the funded period, for example, repository name and link data set(s)
- Additional forms as described in the Notice of Award (e.g., Equipment Inventory Report, Final Invention Statement).

4. Federal Funding Accountability and Transparency Act of 2006 (FFATA)

Federal Funding Accountability and Transparency Act of 2006 (FFATA), P.L. 109–282, as amended by section 6202 of P.L. 110–252 requires full disclosure of all entities and organizations receiving Federal funds including awards, contracts, loans, other assistance, and payments through a single publicly accessible Web site, http://www.USASpending.gov. Compliance with this law is primarily the responsibility of the Federal agency. However, two elements of the law require information to be collected and reported by applicants: 1) information on executive compensation when not already reported through the SAM, and 2) similar information on all sub-awards/subcontracts/consortiums over $25,000.
For the full text of the requirements under the FFATA and HHS guidelines, go to:

- https://www.gpo.gov/fdsys/pkg/PLAW-109publ282/pdf/PLAW-109publ282.pdf,
- https://www. fsrs.gov/documents /ffata_legislation_ 110_252.pdf
- http://www.hhs.gov/grants/grants/grants-policies-regulations/index.html#FFATA.

5. Reporting of Foreign Taxes (International/Foreign projects only)

A. Valued Added Tax (VAT) and Customs Duties – Customs and import duties, consular fees, customs surtax, valued added taxes, and other related charges are hereby authorized as an allowable cost for costs incurred for non-host governmental entities operating where no applicable tax exemption exists. This waiver does not apply to countries where a bilateral agreement (or similar legal document) is already in place providing applicable tax exemptions and it is not applicable to Ministries of Health. Successful applicants will receive information on VAT requirements via their Notice of Award.

B. The U.S. Department of State requires that agencies collect and report information on the amount of taxes assessed, reimbursed and not reimbursed by a foreign government against commodities financed with funds appropriated by the U.S. Department of State, Foreign Operations and Related Programs Appropriations Act (SFOAA) ("United States foreign assistance funds"). Outlined below are the specifics of this requirement:

1) Annual Report: The recipient must submit a report on or before November 16 for each foreign country on the amount of foreign taxes charged, as of September 30 of the same year, by a foreign government on commodity purchase transactions valued at 500 USD or more financed with United States foreign assistance funds under this grant during the prior United States fiscal year (October 1 – September 30), and the amount reimbursed and unreimbursed by

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the foreign government. [Reports are required even if the recipient did not pay any taxes during the reporting period.]

2) Quarterly Report: The recipient must quarterly submit a report on the amount of foreign taxes charged by a foreign government on commodity purchase transactions valued at 500 USD or more financed with United States foreign assistance funds under this grant. This report shall be submitted no later than two weeks following the end of each quarter: April 15, July 15, October 15 and January 15.

3) Terms: For purposes of this clause:
"Commodity" means any material, article, supplies, goods, or equipment;
"Foreign government" includes any foreign government entity;
"Foreign taxes" means value-added taxes and custom duties assessed by a foreign government on a commodity. It does not include foreign sales taxes.

4) Where: Submit the reports to the Director and Deputy Director of the CDC office in the country(ies) in which you are carrying out the activities associated with this cooperative agreement. In countries where there is no CDC office, send reports to VATreporting@cdc.gov.

5) Contents of Reports: The reports must contain:
a. recipient name;
b. contact name with phone, fax, and e-mail;
c. agreement number(s) if reporting by agreement(s);
d. reporting period;
e. amount of foreign taxes assessed by each foreign government;
f. amount of any foreign taxes reimbursed by each foreign government;
g. amount of foreign taxes unreimbursed by each foreign government.

6) Subagreements. The recipient must include this reporting requirement in all applicable subgrants and other subagreements.

G. Agency Contacts

CDC encourages inquiries concerning this notice of funding opportunity.

Program Office Contact

For programmatic technical assistance, contact:

Patrick Chong, Project Officer
Department of Health and Human Services
Centers for Disease Control and Prevention
Division of Global Health Protection

7300 Beijing Place, Box 0051

Washington, DC 20521-7300

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Email: pec8@cdc.gov

Grants Staff Contact

For **financial, awards management, or budget assistance,** contact:

Dionne Bounds, Grants Management Officer, Grants Management Specialist
Department of Health and Human Services
Office of Grants Services
2920 Brandywine Road

Atlanta, GA, 30341-4146

Telephone: 770.488.2082
Email: vhv5@cdc.gov

For assistance with **submission difficulties related to** www.grants.gov, contact the Contact Center by phone at 1-800-518-4726.
Hours of Operation: 24 hours a day, 7 days a week, except on federal holidays.

For all other **submission** questions, contact:
Technical Information Management Section
Department of Health and Human Services
CDC Office of Financial Resources
Office of Grants Services
2920 Brandywine Road, MS E-14
Atlanta, GA 30341
Telephone: 770-488-2700
E-mail: ogstims@cdc.gov

CDC Telecommunications for persons with hearing loss is available at: TTY 1-888-232-6348

H. Other Information

Following is a list of acceptable attachments **applicants** can upload as PDF files as part of their application at www.grants.gov. Applicants may not attach documents other than those listed; if other documents are attached, applications will not be reviewed.

- Project Abstract
- Project Narrative
- Budget Narrative
- CDC Assurances and Certifications
- Report on Programmatic, Budgetary and Commitment Overlap
- Table of Contents for Entire Submission

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For international NOFOs:

- SF424
- SF424A
- Funding Preference Deliverables

https://www.cdc.gov/globalhealth/healthprotection/index.html

I. Glossary

Activities: The actual events or actions that take place as a part of the program.
Administrative and National Policy Requirements, Additional Requirements (ARs): Administrative requirements found in 45 CFR Part 75 and other requirements mandated by statute or CDC policy. All ARs are listed in the Template for CDC programs. CDC programs must indicate which ARs are relevant to the NOFO; recipients must comply with the ARs listed in the NOFO. To view brief descriptions of relevant provisions, see http:// www.cdc.gov/ grants/ additional requirements/ index.html. Note that 2 CFR 200 supersedes the administrative requirements (A-110 & A-102), cost principles (A-21, A-87 & A-122) and audit requirements (A-50, A-89 & A-133).
Approved but Unfunded: Approved but unfunded refers to applications recommended for approval during the objective review process; however, they were not recommended for funding by the program office and/or the grants management office.
Award: Financial assistance that provides support or stimulation to accomplish a public purpose. Awards include grants and other agreements (e.g., cooperative agreements) in the form of money, or property in lieu of money, by the federal government to an eligible applicant.
Budget Period or Budget Year: The duration of each individual funding period within the project period. Traditionally, budget periods are 12 months or 1 year.
Carryover: Unobligated federal funds remaining at the end of any budget period that, with the approval of the GMO or under an automatic authority, may be carried over to another budget period to cover allowable costs of that budget period either as an offset or additional authorization. Obligated but liquidated funds are not considered carryover.
Catalog of Federal Domestic Assistance (CFDA): A government-wide compendium published by the General Services Administration (available on-line in searchable format as well as in printable format as a .pdf file) that describes domestic assistance programs administered by the Federal Government.
CFDA Number: A unique number assigned to each program and NOFO throughout its lifecycle that enables data and funding tracking and transparency.
CDC Assurances and Certifications: Standard government-wide grant application forms.
Competing Continuation Award: A financial assistance mechanism that adds funds to a grant and adds one or more budget periods to the previously established period of performance (i.e., extends the "life" of the award).
Continuous Quality Improvement: A system that seeks to improve the provision of services with an emphasis on future results.
Contracts: An award instrument used to acquire (by purchase, lease, or barter) property or services for the direct benefit or use of the Federal Government.

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Cooperative Agreement: A financial assistance award with the same kind of interagency relationship as a grant except that it provides for substantial involvement by the federal agency funding the award. Substantial involvement means that the recipient can expect federal programmatic collaboration or participation in carrying out the effort under the award.
Cost Sharing or Matching: Refers to program costs not borne by the Federal Government but by the recipients. It may include the value of allowable third-party, in-kind contributions, as well as expenditures by the recipient.
Direct Assistance: A financial assistance mechanism, which must be specifically authorized by statute, whereby goods or services are provided to recipients in lieu of cash. DA generally involves the assignment of federal personnel or the provision of equipment or supplies, such as vaccines. DA is primarily used to support payroll and travel expenses of CDC employees assigned to state, tribal, local, and territorial (STLT) health agencies that are recipients of grants and cooperative agreements. Most legislative authorities that provide financial assistance to STLT health agencies allow for the use of DA. http:// www.cdc.gov /grants /additionalrequirements /index.html.
DUNS: The Dun and Bradstreet (D&B) Data Universal Numbering System (DUNS) number is a nine-digit number assigned by Dun and Bradstreet Information Services. When applying for Federal awards or cooperative agreements, all applicant organizations must obtain a DUNS number as the Universal Identifier. DUNS number assignment is free. If requested by telephone, a DUNS number will be provided immediately at no charge. If requested via the Internet, obtaining a DUNS number may take one to two days at no charge. If an organization does not know its DUNS number or needs to register for one, visit Dun & Bradstreet at http://fedgov.dnb.com/ webform/displayHomePage.do.
Evaluation (program evaluation): The systematic collection of information about the activities, characteristics, and outcomes of programs (which may include interventions, policies, and specific projects) to make judgments about that program, improve program effectiveness, and/or inform decisions about future program development.
Evaluation Plan: A written document describing the overall approach that will be used to guide an evaluation, including why the evaluation is being conducted, how the findings will likely be used, and the design and data collection sources and methods. The plan specifies what will be done, how it will be done, who will do it, and when it will be done. The NOFO evaluation plan is used to describe how the recipient and/or CDC will determine whether activities are implemented appropriately and outcomes are achieved.
Federal Funding Accountability and Transparency Act of 2006 (FFATA): Requires that information about federal awards, including awards, contracts, loans, and other assistance and payments, be available to the public on a single website at www.USAspending.gov.
Fiscal Year: The year for which budget dollars are allocated annually. The federal fiscal year starts October 1 and ends September 30.
Grant: A legal instrument used by the federal government to transfer anything of value to a recipient for public support or stimulation authorized by statute. Financial assistance may be money or property. The definition does not include a federal procurement subject to the Federal Acquisition Regulation; technical assistance (which provides services instead of money); or assistance in the form of revenue sharing, loans, loan guarantees, interest subsidies, insurance, or direct payments of any kind to a person or persons. The main difference between a grant and a cooperative agreement is that in a grant there is no anticipated substantial programmatic involvement by the federal government under the award.

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Grants.gov: A "storefront" web portal for electronic data collection (forms and reports) for federal grant-making agencies at www.grants.gov.
Grants Management Officer (GMO): The individual designated to serve as the HHS official responsible for the business management aspects of a particular grant(s) or cooperative agreement(s). The GMO serves as the counterpart to the business officer of the recipient organization. In this capacity, the GMO is responsible for all business management matters associated with the review, negotiation, award, and administration of grants and interprets grants administration policies and provisions. The GMO works closely with the program or project officer who is responsible for the scientific, technical, and programmatic aspects of the grant.
Grants Management Specialist (GMS): A federal staff member who oversees the business and other non-programmatic aspects of one or more grants and/or cooperative agreements. These activities include, but are not limited to, evaluating grant applications for administrative content and compliance with regulations and guidelines, negotiating grants, providing consultation and technical assistance to recipients, post-award administration and closing out grants.
Health Disparities: Differences in health outcomes and their determinants among segments of the population as defined by social, demographic, environmental, or geographic category.
Health Equity: Striving for the highest possible standard of health for all people and giving special attention to the needs of those at greatest risk of poor health, based on social conditions.
Health Inequities: Systematic, unfair, and avoidable differences in health outcomes and their determinants between segments of the population, such as by socioeconomic status (SES), demographics, or geography.
Healthy People 2020: National health objectives aimed at improving the health of all Americans by encouraging collaboration across sectors, guiding people toward making informed health decisions, and measuring the effects of prevention activities.
Inclusion: Both the meaningful involvement of a community's members in all stages of the program process and the maximum involvement of the target population that the intervention will benefit. Inclusion ensures that the views, perspectives, and needs of affected communities, care providers, and key partners are considered.
Indirect Costs: Costs that are incurred for common or joint objectives and not readily and specifically identifiable with a particular sponsored project, program, or activity; nevertheless, these costs are necessary to the operations of the organization. For example, the costs of operating and maintaining facilities, depreciation, and administrative salaries generally are considered indirect costs.
Intergovernmental Review: Executive Order 12372 governs applications subject to Intergovernmental Review of Federal Programs. This order sets up a system for state and local governmental review of proposed federal assistance applications. Contact the state single point of contact (SPOC) to alert the SPOC to prospective applications and to receive instructions on the State's process. Visit the following web address to get the current SPOC list: http://www.whitehouse.gov/omb/ grants_spoc/.
Letter of Intent (LOI): A preliminary, non-binding indication of an organization's intent to submit an application.
Lobbying: Direct lobbying includes any attempt to influence legislation, appropriations, regulations, administrative actions, executive orders (legislation or other orders), or other similar deliberations at any level of government through communication that directly expresses

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a view on proposed or pending legislation or other orders, and which is directed to staff members or other employees of a legislative body, government officials, or employees who participate in formulating legislation or other orders. Grass roots lobbying includes efforts directed at inducing or encouraging members of the public to contact their elected representatives at the federal, state, or local levels to urge support of, or opposition to, proposed or pending legislative proposals.

Logic Model: A visual representation showing the sequence of related events connecting the activities of a program with the programs' desired outcomes and results.

Maintenance of Effort: A requirement contained in authorizing legislation, or applicable regulations that a recipient must agree to contribute and maintain a specified level of financial effort from its own resources or other non-government sources to be eligible to receive federal grant funds. This requirement is typically given in terms of meeting a previous base-year dollar amount.

Memorandum of Understanding (MOU) or Memorandum of Agreement (MOA): Document that describes a bilateral or multilateral agreement between parties expressing a convergence of will between the parties, indicating an intended common line of action. It is often used in cases where the parties either do not imply a legal commitment or cannot create a legally enforceable agreement.

Nonprofit Organization: Any corporation, trust, association, cooperative, or other organization that is operated primarily for scientific, educational, service, charitable, or similar purposes in the public interest; is not organized for profit; and uses net proceeds to maintain, improve, or expand the operations of the organization. Nonprofit organizations include institutions of higher educations, hospitals, and tribal organizations (that is, Indian entities other than federally recognized Indian tribal governments).

Notice of Award (NoA): The official document, signed (or the electronic equivalent of signature) by a Grants Management Officer that: (1) notifies the recipient of the award of a grant; (2) contains or references all the terms and conditions of the grant and Federal funding limits and obligations; and (3) provides the documentary basis for recording the obligation of Federal funds in the HHS accounting system.

Objective Review: A process that involves the thorough and consistent examination of applications based on an unbiased evaluation of scientific or technical merit or other relevant aspects of the proposal. The review is intended to provide advice to the persons responsible for making award decisions.

Outcome: The results of program operations or activitIes; the effects triggered by the program. For example, increased knowledge, changed attitudes or beliefs, reduced tobacco use, reduced morbidity and mortality.

Performance Measurement: The ongoing monitoring and reporting of program accomplishments, particularly progress toward pre-established goals, typically conducted by program or agency management. Performance measurement may address the type or level of program activities conducted (process), the direct products and services delivered by a program (outputs), or the results of those products and services (outcomes). A "program" may be any activity, project, function, or policy that has an identifiable purpose or set of objectives.

Period of performance –formerly known as the project period - : The time during which the recipient may incur obligations to carry out the work authorized under the Federal award. The start and end dates of the period of performance must be included in the Federal award.

Period of Performance Outcome: An outcome that will occur by the end of the NOFO's

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funding period

Plain Writing Act of 2010: The Plain Writing Act of 2010 requires that federal agencies use clear communication that the public can understand and use. NOFOs must be written in clear, consistent language so that any reader can understand expectations and intended outcomes of the funded program. CDC programs should use NOFO plain writing tips when writing NOFOs.

Program Strategies: Strategies are groupings of related activities, usually expressed as general headers (e.g., Partnerships, Assessment, Policy) or as brief statements (e.g., Form partnerships, Conduct assessments, Formulate policies).

Program Official: Person responsible for developing the NOFO; can be either a project officer, program manager, branch chief, division leader, policy official, center leader, or similar staff member.

Public Health Accreditation Board (PHAB): A nonprofit organization that works to promote and protect the health of the public by advancing the quality and performance of public health departments in the U.S. through national public health department accreditation http://www.phaboard.org.

Social Determinants of Health: Conditions in the environments in which people are born, live, learn, work, play, worship, and age that affect a wide range of health, functioning, and quality-of-life outcomes and risks.

Statute: An act of the legislature; a particular law enacted and established by the will of the legislative department of government, expressed with the requisite formalities. In foreign or civil law any particular municipal law or usage, though resting for its authority on judicial decisions, or the practice of nations.

Statutory Authority: Authority provided by legal statute that establishes a federal financial assistance program or award.

System for Award Management (SAM): The primary vendor database for the U.S. federal government. SAM validates applicant information and electronically shares secure and encrypted data with federal agencies' finance offices to facilitate paperless payments through Electronic Funds Transfer (EFT). SAM stores organizational information, allowing www.grants.gov to verify identity and pre-fill organizational information on grant applications.

Technical Assistance: Advice, assistance, or training pertaining to program development, implementation, maintenance, or evaluation that is provided by the funding agency.

Work Plan: The summary of period of performance outcomes, strategies and activities, personnel and/or partners who will complete the activities, and the timeline for completion. The work plan will outline the details of all necessary activities that will be supported through the approved budget.

NOFO-specific Glossary and Acronyms

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From: Folkers, Greg (NIH/NIAID) [E]
Sent: Tue, 24 Apr 2018 11:31:26 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: RE: NBC.com/Fauci+Routh: Flu season is finally ending, but the race to create next year's flu vaccine is just beginning https://nbcnews.to/2HKo4VI

Yes

Will do
Also director page

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Tuesday, April 24, 2018 7:31 AM
To: Folkers, Greg (NIH/NIAID) [E] (b)(6)
Subject: FW: NBC.com/Fauci+Routh: Flu season is finally ending, but the race to create next year's flu vaccine is just beginning https://nbcnews.to/2HKo4VI

Should this go on the new part of the CV that included lay newspaper editorials and articles?

From: Folkers, Greg (NIH/NIAID) [E]
Sent: Tuesday, April 24, 2018 7:27 AM
Subject: NBC.com/Fauci+Routh: Flu season is finally ending, but the race to create next year's flu vaccine is just beginning https://nbcnews.to/2HKo4VI

Flu season is finally ending, but the race to create next year's flu vaccine is just beginning

Despite substantial advances in science, we are still vulnerable to severe influenza seasons and devastating pandemics. We need a universal flu vaccine.

by Dr. Anthony S. Fauci and Jennifer M. Routh / Apr.24.2018 / 4:39 AM ET

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Research shows that seasonal influenza vaccines are approximately 10 to 60 percent effective depending on the year.Brian Snyder / Reuters

When it comes to infectious diseases, we must always be prepared for the unexpected. From the emergence of HIV/AIDS decades ago to the recent spread of Zika virus across the Americas, we continually see unknown diseases emerge and familiar diseases unpredictably re-emerge. Influenza, however, has remained a consistent threat for centuries.

As this year's flu season winds down, manufacturers already are preparing updated vaccines for the upcoming flu season this fall and winter. While seasonal flu vaccines are important public health tools, our current approach can leave us one step behind the ever-changing influenza virus. Therefore, scientists at academic institutions, private companies and here at the National Institute of Allergy and Infectious Diseases (NIAID) are working to develop a universal influenza vaccine that would protect us against whatever flu strains that emerge.

Related FLU FRENZY Why is the flu such a big killer?

Anyone who has caught the flu knows it can be a miserable experience. On a broader scale, seasonal influenza causes a substantial public health burden, and the potential for a new virus strain to emerge and cause a pandemic remains a likely possibility. Each year in the United States, seasonal influenza leads to between 140,000 and 710,000 hospitalizations and as many as 56,000 deaths.

The 2017-2018 U.S. influenza season has been particularly severe with high rates of hospitalizations and 156 influenza-associated pediatric deaths as of April 14, 2018. This year also marks the 100th anniversary of the 1918 influenza pandemic, during which one-third of the world's population was infected with influenza and at least 50 million individuals died.

Despite substantial advances in science and medicine, we are still vulnerable to severe influenza seasons and devastating pandemics. Although it is always better to get the seasonal influenza vaccine than not, we must acknowledge the limitations of our current vaccines. Year after year, seasonal influenza vaccines have offered suboptimal protection, especially when compared to licensed vaccines for other infectious diseases.

Research shows that seasonal influenza vaccines are approximately 10 to 60 percent effective depending on the year. By contrast, two doses of the measles vaccine are approximately 97 percent effective, and do not require annual vaccination. In addition, seasonal influenza vaccines would offer virtually no protection against pandemic influenza strains.

A more protective and durable influenza vaccine has the potential to further reduce hospitalization rates and save thousands of lives annually. However, influenza poses unique scientific and logistical challenges. Influenza viruses constantly mutate, or drift, in small but important ways, which is why

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influenza vaccines are updated each year to best match the virus strains projected to circulate in the next season.

The cycle of updating the vaccine for the upcoming winter influenza season begins every February-March in the United States and requires a months-long manufacturing process that most commonly involves growing influenza viruses in eggs. By the time the U.S. influenza season begins in the late fall, mutated viruses can become more widespread than other circulating strains. Because the vaccine was not designed to protect against these mutated strains, it can be less effective.

The cycle of updating the vaccine for the upcoming winter influenza season begins every February-March in the United States and requires a months-long manufacturing process.

Research also suggests that the egg-based production process itself can lead to small changes in the vaccine virus that can contribute to low vaccine effectiveness. Other factors, such as a person's age, their co-existing health conditions and their prior influenza exposures and vaccinations also impact how well influenza vaccines work.

Influenza viruses also can undergo more profound changes in composition at any time, resulting in a virus that is dramatically different from those that have circulated previously. Under such circumstances, few or no people would have immunity to the new form of the virus, leading to a pandemic. This scenario unfolded most recently in 2009 with the introduction of a new H1N1 influenza virus.

It is very likely that another influenza pandemic will occur eventually, although it is impossible to predict when it will happen and how severe it will be. As part of a larger preparedness effort for such an occurrence, the U.S. government develops and stockpiles vaccines against "pre-pandemic" influenza virus strains, animal viruses that occasionally infect humans, but do not yet spread efficiently among people.

Related IT'S A PROCESS Next year's flu vaccine will be lousy, too

There is a great deal of uncertainty to this approach since it is difficult to predict precisely which viruses may mutate to become easily transmittable. Also, like seasonal influenza viruses, these animal viruses may mutate after vaccines are produced, and new vaccines would need to be developed. This continually positions us one step behind the virus.

Not surprisingly, the limitations of seasonal influenza vaccines and the threat of an influenza pandemic have driven an extensive effort in the scientific community to develop a universal influenza vaccine — a vaccine that can provide durable protection for all age groups against multiple strains, including those that might cause a pandemic.

NIAID convened a workshop in June 2017 that gathered scientists from academia, industry and government to develop criteria for defining such a universal influenza vaccine, to identify knowledge gaps in influenza research, and to discuss strategies for addressing those gaps. Based on the deliberations of that meeting, NIAID recently issued a strategic plan to set a universal influenza vaccine research agenda.

Not surprisingly, the limitations of seasonal influenza vaccines and the threat of an influenza pandemic have driven an extensive effort to develop a universal vaccine.

One leading approach involves inducing an immune response against a part of the influenza virus that does not change from year to year. Although this may seem simple, it is actually a complicated effort because the immune system tends to respond to the part of the influenza virus that does change. Advances in virology, immunology and vaccinology make this endeavor more feasible than it was a decade ago. However, scientists are still working to better understand how influenza transmits, what exactly makes certain infections more severe than others and how someone's prior bouts of influenza or previous vaccinations could affect future infections, among other research questions

Overcoming these scientific challenges to create a universal vaccine will take time, but we believe it is possible. In the meantime, NIAID will continue to get our flu vaccine every year and encourage everyone to do the same. Some protection against influenza infection is better than none, and research shows

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that it can reduce your chances of severe illness and death if you do happen to catch this notorious virus.

Anthony S. Fauci, M.D., is director of the National Institute of Allergy and Infectious Diseases (NIAID), part of NIH. Jennifer M. Routh is an NIAID science writer/editor.

Disclaimer: Any third-party material in this email has been shared under fair use provisions of U.S. copyright law, without further verification of its accuracy/veracity. It does not necessarily represent my views nor those of NIAID, NIH, HHS, or the U.S. government.

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From: Folkers, Greg (NIH/NIAID) [E]
Sent: Tue, 24 Apr 2018 01:51:16 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: RE: THINK PRE-PITCH for TUESDAY at 430am: Dr. Anthony S. Fauci: The race to stop next year's flu deaths starts now. And we're already behind.

That piece on flu u wrote (with Jen Routh) is being posted by NBC.com, and apparently they are telling their reporters/stations about it, I guess so they can start calling u….

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Monday, April 23, 2018 9:49 PM
To: Folkers, Greg (NIH/NIAID) [E] (b)(6)
Subject: FW: THINK PRE-PITCH for TUESDAY at 430am: Dr. Anthony S. Fauci: The race to stop next year's flu deaths starts now. And we're already behind.

What is this???

From: Fox, Maggie (NBCUniversal) (b)(6)
Sent: Monday, April 23, 2018 8:28 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: THINK PRE-PITCH for TUESDAY at 430am: Dr. Anthony S. Fauci: The race to stop next year's flu deaths starts now. And we're already behind.

Oh good!

Maggie Fox
Senior Writer, NBC News
@maggiemfox
www.nbcnews.com/health

Subject: THINK PRE-PITCH for TUESDAY at 430am: Dr. Anthony S. Fauci: The race to stop next year's flu deaths starts now. And we're already behind.

Article: Flu season is finally ending, but the race to create next year's flu vaccine is just beginning

Cover: The race to stop next year's flu deaths starts now. And we're already behind.

Social: Opinion | Dr. Anthony S. Fauci: The race to counter next year's flu starts now (and we're behind)

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From: Eisinger, Robert (NIH/NIAID) [E]
Sent: Mon, 23 Apr 2018 22:18:18 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Doepel, Laurie (NIH/NIAID) [E];Folkers, Greg (NIH/NIAID) [E]
Subject: FW: Lancet Commission final pre-submission draft Review
Attachments: Lancet TB Report Comments 4.23.18.docx, Spectrum of TB Research and Development NIAID 12.19.17.pdf

Draft email Mike Reid ((b)(6) and Eric Goosby (b)(6)

Eric and Mike

Thank you for the opportunity to review the entire draft Report. Your efforts to compile the feedback from all of the Commissioners into this draft should be applauded.

The first attachment labeled "Lancet TB Report Comments 4.23.18" lists significant comments and suggested edits in each section. I wanted to bring your attention a few of the most important of these, highlighted below:

- Executive Summary - Vision Statement – I would propose the following: "With bold leadership, accelerated research and development, implementation of evidence-based strategies, and a shared responsibility, we can end TB within a generation."
- Executive Summary - Priority 1 should be revised to include as the next to the last sentence "Special attention should be focused on TB and HIV coinfection."
- Executive Summary - Table 1 should be used rather than Figure 1.
- Section 2 – I propose an additional priority for this Section should be added -- "Invest in critical research on TB and HIV coinfection."
- Section 2 – Figure 2.0.1 should be used and not Figure 2.2.1. Two modifications are needed to Figure 2.0.1 including: 1) the "Industry and Academic Partnerships" bar on the schematic needs to be larger reflecting its importance in the product development process; and 2) this bar on the schematic needs to be narrower in the "Basic Science/Biomarker" region and gradually get wider. The attached schematic which was provided previously illustrates the latter modification.

Additional comments and suggested edits can be found in each section of the Report in the tracked changes versions at the links below. As noted, in a few instances, figures/tables cited in the narrative are missing, or figures/tables that appear in the report are not cited in the narrative.

-

Executive Summary link https://file.ac/4vvxNEHLGyY/
Section 0 link https://file.ac/e4dGSd7FYVE/
Section 1 link https://file.ac/lpG4ovDsWYw/
Section 2 link https://file.ac/xQCyttQzkjE/
Section 3 link https://file.ac/D4R94Fn5hjk/
Section 4 link https://file.ac/Ue7pZHn3PoY/

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Panels link https://file.ac/eJRGAfikEic/

I suggest that the non-Commissioners who assisted in developing the Report should be acknowledged but not designated as co-authors. -

If we can provide any additional information or assistance, please let us know.
Best,

Tony

---

rom: Reid, Michael (b)(6)
Sent: Friday, April 20, 2018 3:14 AM
To: Goosby, Eric (b)(6)
Cc: Dean Jamison (b)(6) Soumya Swaminathan (b)(6)
Subject: Re: Lancet Commission final pre-submission draft Review

Dear Commissioners,

Thank you to all those of you that have contributed to revising the report and responding to the external reviewer comments. In particular, thanks to the teams from NIH and FIND for their help pulling pieces together!

Please find attached a zip file containing the current draft [minus a few pending analyses]. The full report – in 6 sections – can also be downloaded:

From Box: https://ucsf.box.com/s/uqzzpsza4w86x2rmulvyua0bufylpsgi

From Dropbox: https://www.dropbox.com/sh/9se9699agk5s5ck/AAAXQKMeELK728MoCzm-Oifua?dl=0

In order to ensure that we have the best chance of getting the report published in time for the HLM, The Lancet have requested that we formally submit by **April 27th**. I fully appreciate that this means everyone has a short time to review and respond. Nonetheless, we would be grateful for your comments/edits by **April 24** if possible.

In particular, please review the executive summary, in particular the 'vision statement' and the more concise recommendations (5 rather than 10), which align with the HLM 'key asks'.

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Eric and I welcome your feedback via email. If possible, share any substantive recommendations/comments in the email, rather than track changes to the documents, to ensure we don't miss any major comments. However, feel free to use track changes if that is your preference. I can also be available most of next week (April 23-26) by 'phone or Skype to discuss questions/clarifications/concerns.

Finally, several of you partnered with non-commissioners to complete writing/analytic products – If you feel these colleagues should be considered as co-authors on the publication, *and you haven't already reached to me to discuss*, please let me know.

Thank you all for your continued support and collaboration in this effort and the important roles you each play in 'building a TB-free world.'

Best regards,

Mike

Lancet Commission on TB, Secretariat

From: "Reid, Michael" (b)(6)
Date: Sunday, April 15, 2018 at 06:22
To: Eric Goosby (b)(6)
Subject: Re: Lancet Commission Conf calls - week of April 16-20

Dear Commissioners,

Apologies for the delay in failing to circle back on this email from David sooner. Unfortunately, we were unable to find any date/time that worked for more than 2 or 3 people. With this in mind, Eric and I have decided to forgo a conference call for all Commissioners.

We are aiming to formally submit a revised draft of the Commission, as well as responses to all the external reviewers' comments, to The Lancet by **April 27**. In advance of that date, I intend to share a pre-submission draft of the full report to all Commissioners this coming Wed/Thurs [**April 18/19**]. I kindly ask that you all take time to review and make edits/comments – sending back any comments/edits no later than April 23.

I can personally be available for one-on-one calls on April 23 and 24 for anyone that wants to give feedback over the 'phone. Please email me individually if you would like to connect on either day.

Thanks for all of your input, particularly those of you that have been re-writing or reviewing subsections of the report over the last 2 weeks.

Kind regards,

Mike Reid

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(on behalf of the Commission Secretariat)

From: David McKey (b)(6)
Date: Wednesday, April 4, 2018 at 09:52
To: David McKey (b)(6)
Cc: "Reid, Michael" (b)(6)
Subject: Lancet Commission Conf calls - week of April 16-20

Dear Commissioners,
As a follow-up to our recent meeting in New Delhi, it was agreed that we would arrange for two conference calls the week of April 16-20 to give an update on the progress and discuss any last minute details before the report is submitted the end of April.

Please find the link for a doodle poll indicating possible times for a call. Please select the two dates and times that work the best for you.
(b)(6)

If you plan on submitting expenses for reimbursement, please send as soon as possible. As an FYI, I will be out on holiday 10-22 April. Expenses not received before 6 April will be submitted upon my return to the office.

And, attached is the picture from our meeting.

Thank you,
David

David McKey
Office of the UN Secretary-General's Special Envoy on Tuberculosis
Global Health Delivery & Diplomacy
Institute for Global Health Sciences | UCSF
550 16th Street | 3rd Floor | Box 1224
San Francisco, California 94158

(b)(6)
(b)(6)

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UCSF Institute for Global Health Sciences

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Issues/Modifications for Lancet Commission Report (version 4.19.18)

Executive Summary (Recommendations/Priorities):

Lines 16-21 Vision Statement modification proposed: "With bold leadership, accelerated research and development, implementation of evidence-based strategies, and a shared responsibility, we can end TB within a generation."

Line 28 Delete comparison of priority on TB vs. HIV and Malaria.

Lines 49-50 Delete sentence citing the inadequate pace of TB R&D helped fuel DR-TB.

Line 110 Cites 3 million lives saved in Kenya; however, the narrative on Fig. 1C shows 4M and the graphic indicates 3M.

Lines 192-193 Priority 1 should be modified to include "Special attention should be focused on TB and HIV coinfection."

Figure 1 or Table 1 – Table 1 should be used rather than Figure 1. In either case, one needs to confirm that number of deaths averted in Kenya is 3M not 4M as cited above for Line 110.

Section 0 (Report Roadmap/Overview):

Figures 0.5.1 and 0.5.2 are not mentioned in the narrative but are included in this Section.

Line 126 Please clarify what the term "pro-poor" means.

Line 136 Please clarify what the phrase "protecting the TB risk pool" means.

Line 156-182 Confirm/add/update sources, dates, and data as needed for TB mortality and incidence. Some data through 2016 are from 2017 Global TB Report, other text references 2000-2015 data from Table 1.

Line 179 Table 0.4 is not found in this Section.

Line 247 A number needs to be assigned to Figure 0.x

Line 252 Needs to be corrected to 45% so that is matches the data in Fig. 0.4.

Line 258 Need to define NTP (National Tuberculosis [TB] Programme).

Line 268 Table 0.x is not included in this Section.

Section 1 (Strategies to reduce TB mortality):

Figures 1.3.1 There are two figures with this number.

Figures 1.5.1 There are two figures with this number.

Tables 1.3.2 There are two Tables with this number.

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Consider eliminating the Recommendations at the end of each subsection and fold any essential information they contain into the overall Recommendations at the beginning of Section 1. Subsection Recommendations lengthen Section 1 considerably and do not appear in other Sections.

Lines 62-77 Several priorities discussed in Section 1 are not included in the summary of policy priorities. Include them in first bullet or create a separate bullet to address. These priorities include establishing patient-centered care, closing the gaps in the TB care continuum, and instituting quality management.

Line 261-262 Please clarify "through peer exchange through international platforms for knowledge management."

Line 321 Please clarify/define the phrase "high-risk pools".

Line 357 There are two Figures labeled 1.3.1.

Lines 384-386 Please confirm this statement.

Line 553 Where is Figure 1?

Line 625 Where is Table 1.3.3?

Line 695 The title of the sub-section is not aligned with the focus of the narrative that follows.

Line 729 There are two Figures labeled 1.5.1, but this sentence matches with the first Figure, so it should be numbered 1.5.0.

Page 58-61 What narrative does this match with?

Section 2 (Research and Development):

Figure 2.2.3 Where does this match with the narrative?

Lines 25-35 In addition to the priorities provided in this section, an additional priority is "Invest in critical research on TB and HIV coinfection."

Line 49 Figure 2.0.1 should be included in the report. A slight modification to this Figure is proposed to accurately show that the "Industry and Academic Partnerships" bar on this schematic is larger; however, this component of the pipeline should begin gradually during the Basic Science/Biomarker Stage of the pipeline and then get larger as one moves down the pipeline. This gradual increase was reflected in the Schematic previously provided to Mike Reid and attached to the transmittal email.

Figure 2.2.1 should not be included in the report.

Section 3 (Macroeconomics):

Line 35 What is supposed to be included in this bullet?

Line 94 and 141 Please clarify/define the term "firms".

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Line 227 Delete sentence that compares funding for TB vs. HIV and malaria.

Line 263 Please clarify/define the term "pro-poor".

Section 4 (Enabling Strategies and Accountability):

Line 50 Please clarify/define the "four domains".

Line 128 Please clarify/define the term "built".

Lines 175-177 This sentence is awkward and needs to be rewritten.

Line 249 refers to Table 4.9.2, but it is not located in this section.

Panels (Background on Specific Topics):

Panel 2, #1. Please use a different term than "infantilize".

Panel 3, Replace "undiagnosed" for the term "missed".

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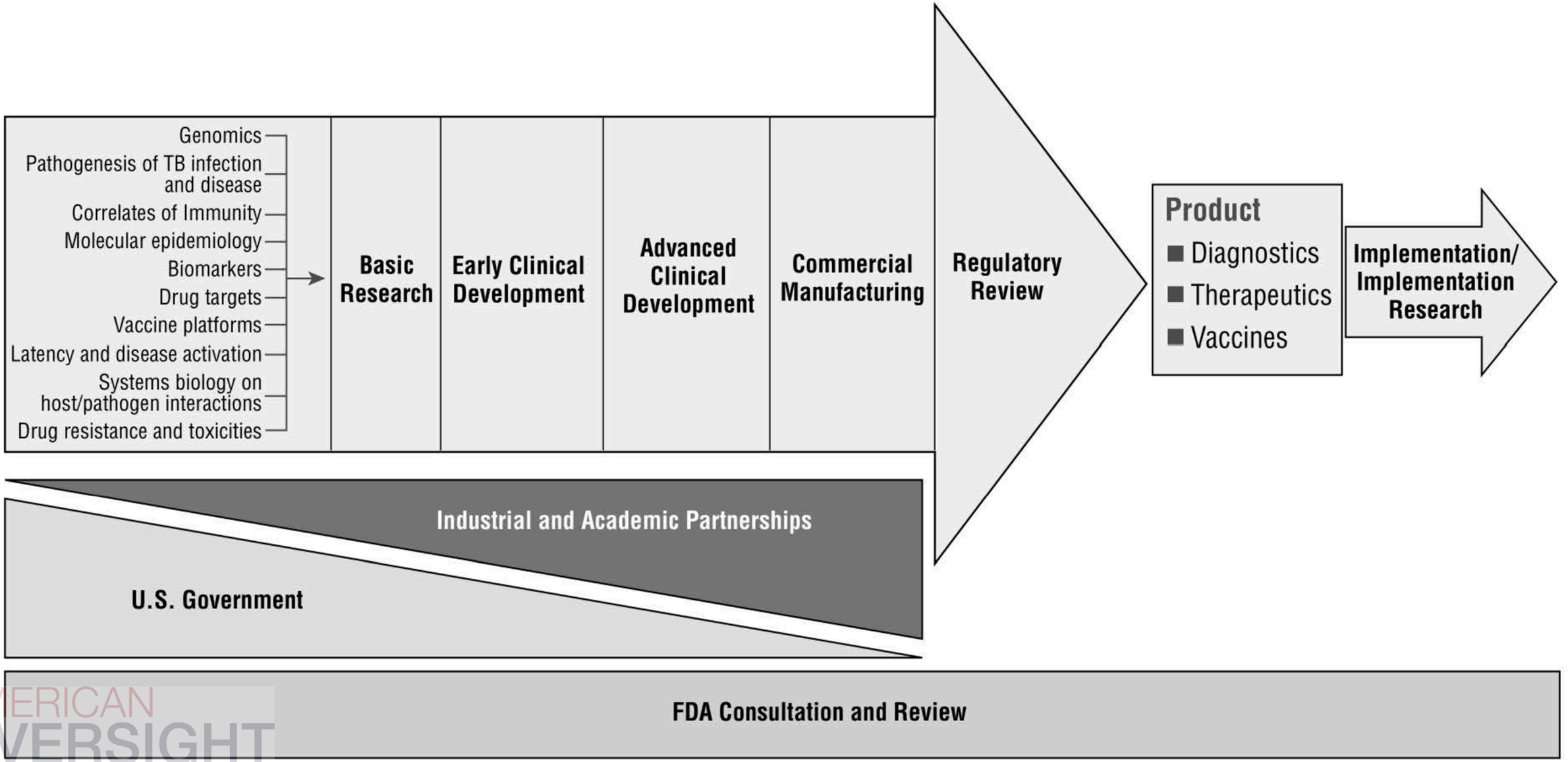

NIH/NIAID, 2017

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Mon, 23 Apr 2018 17:49:51 +0000
To: Fauci, Anthony (NIH/NIAID) [E];Haskins, Melinda (NIH/NIAID) [E];Crawford, Chase (NIH/NIAID) [E];Selgrade, Sara (NIH/NIAID) [E]
Subject: Pre-brief for senate approps clerks visit
Attachments: RE: Please advise: AgendaSenateClerks051118

AMERICAN OVERSIGHT

From: Selgrade, Sara (NIH/NIAID) [E]
Sent: Mon, 23 Apr 2018 17:29:09 +0000
To: Vance, Meaghan (NIH/NIAID) [C];Conrad, Patricia (NIH/NIAID) [E]
Cc: NIAID OCGR Leg;Folkers, Greg (NIH/NIAID) [E]
Subject: RE: Please advise: AgendaSenateClerks051118

Thanks Meaghan. Can we do the prebrief on April 26th at 11am?

Thanks very much for your help,
Sara

From: Vance, Meaghan (NIH/NIAID) [C]
Sent: Monday, April 23, 2018 11:25 AM
To: Selgrade, Sara (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: NIAID OCGR Leg (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6)
Subject: RE: Please advise: AgendaSenateClerks051118

ASF is still available at that time on May 11th

He has the following times available for a pre-brief this week:

April 25th 11:30am
April 26th 11-12:30pm
April 27th 2:30pm or 4pm

Will any of these options work?

Thanks!
Meaghan

From: Selgrade, Sara (NIH/NIAID) [E]
Sent: Monday, April 23, 2018 10:56 AM
To: Conrad, Patricia (NIH/NIAID) [E] (b)(6)
Cc: NIAID OCGR Leg (b)(6) Vance, Meaghan (NIH/NIAID) [C] (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6)
Subject: Please advise: AgendaSenateClerks051118

Hi Patty,

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(b)(6) has let us know the proposed agenda for the May 11 visit of the Senate appropriations clerks (attached). They would like him to meet with the clerks from 1:05 – 1:45 pm in (b)(6) (b)(6) Topics are AMR and flu.

Can you confirm he would be available at this time? And would it be possible to schedule a 30-minute pre-brief with him later this week? Melinda, Chase, and I will participate.

Thanks much for your help,
Sara

Sara Selgrade, Ph.D.
Section Chief for Legislative Activities
Legislative Affairs and Correspondence Management Branch
Office of Communications and Government Relations
NIAID/NIH/DHHS
Bldg. 31, Room 7A17, MSC 2520
Bethesda, MD 20892-2520
Phone: (b)(6)
(b)(6)

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From: Houser, Anne (NIH/OD) [E]
Sent: Monday, April 23, 2018 9:32 AM
To: Aragon, Richard (NIH/NIGMS) [E] (b)(6) Garbarini, Nicole (NIH/OD) [E] (b)(6) Reuss, Reaya (NIH/NIAMS) [E] (b)(6) Norris, Andrea (NIH/CIT) [E] (b)(6) Stakem, Maggi (NIH/CC/OD) [E] (b)(6) Cene, Jessica (NIH/CC/OD) [E] (b)(6) Kelley, Melinda (NIH/NIA/ERP) [E] (b)(6) Beraud, Dawn (NIH/NIA/ERP) [E] (b)(6) NIAID OCGR Leg (b)(6) Blome, Juliana (NIH/OD) [E] (b)(6) Williams, Natasha (NIH/NIMHD) [E] (b)(6) Rieff, Heather (NIH/NINDS) [E] (b)(6) Scott, Paul (NIH/NINDS) [E] (b)(6)
Cc: Klimczak, Kate (NIH/OD) [E] (b)(6) Houser, Anne (NIH/OD) [E] (b)(6) Higgins, Lauren (NIH/OD) [E] (b)(6)
Subject: AgendaSenateClerks051118

Attached is the agenda for the Clerks Visit to NIH on Friday, May 11, 2018. Please look over the times and make sure they work for your speakers. I tried to make sure I put everyone in a time slot for which they were available, and this is the only day they will be on campus. Please put these times and meeting sites on the calendars of the principals. I thank you all for getting back to me to make this possible, especially with all the changes that were made by our visitors. I will be in touch with specifics and options for a call for all of us, in advance of this meeting.

As you prepare, a couple of things to keep in mind (requests from the Clerks):

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- Have a tight agenda for your specific session that you can hand out to the Clerks. And I suggest you time it to be certain half is for presentation and half is for their questions. Please bring paper about everything you discuss. They want paper—they want to have to take with them, specific things you are going to mention—data, timelines, decisions, etc.
- Tell your presenters that there should not be an opening question to the Clerks asking what they are interested in. They have given us the list of everything they are interested in and want that to be addressed right up front.
- If they ask a question about something else, respond but don't lose sight of the original 'ask.'
- Again, thank you so much for helping me on this.

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From: Folkers, Greg (NIH/NIAID) [E]
Sent: Mon, 23 Apr 2018 21:22:48 +0000
To: Fauci, Anthony (NIH/NIAID) [E];Folkers, Greg (NIH/NIAID) [E];Stover, Kathy (NIH/NIAID) [E];Billet, Courtney (NIH/NIAID) [E]
Subject: RE: (for review) AJC media / universal flu
Attachments: The Universal Flu Vaccine.docx

Just a couple minor notes

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The Universal Flu Vaccine

The AJC recently interviewed NIH's Director of the National Institute of Allergy and Infectious Diseases, Dr. Anthony Fauci, who has ~~designating~~ designated a universal flu vaccine to be a top priority. Last summer, he brought together more than 150 leading researchers to map a path. Here, he answers key questions about a universal flu vaccine.

What is a universal flu vaccine?

A universal vaccine would offer robust, long-term protection against influenza.

One approach to designing a universal vaccine is to generate antibody responses to parts of the virus that are common to all influenza strains that don't readily change during mutation.

Fauci said imagine a head of broccoli with a stalk. The head is the part that mutates each season — and it's also the part the current flu vaccine targets.

A new universal vaccine would go deeper, aiming at the interior "stalk" of the virus, which doesn't change season to season.

Why is a universal flu vaccine needed?

Even an ordinary seasonal flu epidemic will kill some 12,000 to 56,000 people every year in the U.S. alone. That is because seasonal viruses continually evolve and mutate, and although we update our vaccines frequently, they may be only 40 to 60 percent effective. This past season, the flu vaccine was only 36 percent effective, and only 25 percent effective against H3N2 influenza, which is causing most flu cases this year.

The goal of the universal flu vaccine would be to offer protection closer to 90 percent for several years with one flu shot.

Where are we in the development and when might we see a universal flu vaccine hit the market?

Several pharmaceutical companies are working on a universal flu vaccine. Researchers at National Institute of Allergies and Infectious Diseases are also working on a universal flu vaccine.

Research is underway at academia, biotech firms and NIH. A handful of major pharmaceutical companies are supporting universal flu vaccine research, including GlaxoSmithKline; Janssen Vaccines, a division of Johnson & Johnson; and Sanofi.

A universal flu vaccine, but only version 1.0, is still at least 5 years a-way. A really effective universal flu vaccine that could provide protection against virtually all strains instead of a select few is at least a decade away.

What do you we do while we wait?

About the current effectiveness rate of flu vaccines, Fauci said, "it is absolutely not good enough."

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That said, he said, "some protection is better than no protection. Even, 20, 30 or 40 percent protection is better than no protection at all."

(I didn't get this from Dr. Fauci. I would like include in this box format, but since I didn't get this from him, I can separate this out or change):

Research is underway at academia, biotech firms and NIH. A handful of major pharmaceutical companies are supporting universal flu vaccine research, including GlaxoSmithKline; Janssen Vaccines, a division of Johnson & Johnson; and Sanofi.

Commented [gkf1]: There are many other players in this "space"

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From: Folkers, Greg (NIH/NIAID) [E]
Sent: Mon, 23 Apr 2018 20:57:19 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: FW: (for review) AJC media / universal flu

They need today
It look ok to me

From: Pekoc, Ken (NIH/NIAID) [E]
Sent: Monday, April 23, 2018 3:15 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6) Folkers, Greg (NIH/NIAID) [E] (b)(6)
Cc: Stover, Kathy (NIH/NIAID) [E] (b)(6) Billet, Courtney (NIH/NIAID) [E] (b)(6)
Subject: (for review) AJC media / universal flu

FYI …

From: Oliviero, Helena (CMG-Atlanta) (b)(6)
Sent: Monday, April 23, 2018 12:52 PM
To: Pekoc, Ken (NIH/NIAID) [E] (b)(6)
Subject: RE: AJC media /REVIEW THIS ONE

Hi Ken,
Thank you for your help interviewing Dr. Fauci.
I have some good quotes for main story but this is for a sidebar box.
I think it is all OK. Could you give it a quick read over or ask Dr. Fauci to give it a quick read over to make sure I got it right?
If any changes are needed, please let me know.
Deadline is end of today.
Thank you!
Helena

The Universal Flu Vaccine

The AJC recently interviewed NIH's Director of the National Institute of Allergy and Infectious Diseases, Dr. Anthony Fauci, who has designating a universal flu vaccine a top priority. Last summer, he brought together more than 150 leading researchers to map a path. Here, he answers key questions about a universal flu vaccine.

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A universal vaccine would offer robust, long-term protection.

One approach to designing a universal vaccine is to generate antibody responses to parts of the virus that are common to all influenza strains that don't readily change during mutation.

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Fauci said imagine a head of broccoli with a stalk. The head is the part that mutates each season — and it's also the part the current flu vaccine targets.

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A universal flu vaccine, but only version 1.0, is still at least 5 years a way. A really effective universal flu vaccine that could provide protection against virtually all strains instead of a select few is at least a decade away.

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About the current effectiveness rate of flu vaccines, Fauci said, "it is absolutely not good enough."

That said, he said, "some protection is better than no protection. Even, 20, 30 or 40 percent protection is better than no protection at all."

(I didn't get this from Dr. Fauci. I would like include in this box format, but since I didn't get this from him, I can separate this out or change):

Research is underway at academia, biotech firms and NIH. A handful of major pharmaceutical companies are supporting universal flu vaccine research, including GlaxoSmithKline; Janssen Vaccines, a division of Johnson & Johnson; and Sanofi.

AMERICAN OVERSIGHT

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AMERICAN OVERSIGHT

From: Folkers, Greg (NIH/NIAID) [E]
Sent: Mon, 23 Apr 2018 20:18:56 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: FW: interview request: Zika HCT/NPR Orlando

fyi

From: Routh, Jennifer (NIH/NIAID) [E]
Sent: Monday, April 23, 2018 3:59 PM
To: Folkers, Greg (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6) Doepel, Laurie (NIH/NIAID) [E] (b)(6) Billet, Courtney (NIH/NIAID) [E] (b)(6) Stover, Kathy (NIH/NIAID) [E] (b)(6)
Cc: Oplinger, Anne (NIH/NIAID) [E] (b)(6) Pekoc, Ken (NIH/NIAID) [E] (b)(6) Deatrick, Elizabeth (NIH/NIAID) [C] (b)(6)
Subject: RE: interview request: Zika HCT/NPR Orlando

FYI – Reporter seemed fine with my suggestion to talk to Seema.

Jennifer Routh [E]
News and Science Writing Branch
Office of Communications and Government Relations
National Institute of Allergy and Infectious Diseases (NIAID)
NIH/HHS
31 Center Drive Room 7A17C
Bethesda, MD 20892
Direct: (b)(6)
(b)(6)

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Monday, April 23, 2018 2:17 PM
To: Folkers, Greg (NIH/NIAID) [E] (b)(6) Routh, Jennifer (NIH/NIAID) [E] (b)(6) Conrad, Patricia (NIH/NIAID) [E] (b)(6) NIAID OD AM (b)(6)
Cc: NIAID COGCORE (b)(6) NIAID Media Inquiries (b)(6)
Subject: RE: interview request: Zika HCT/NPR Orlando

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AMERICAN OVERSIGHT

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(b)(6)
Subject: RE: interview request: Zika HCT/NPR Orlando

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She can do this with no homework.
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And if the reported decides to do a story for broadcast, ASF could be our voice at that point.

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(b)(6)
Subject: interview request: Zika HCT/NPR Orlando

Danielle Prieur (pronounced Pryor)
WMFE radio (NPR), Orlando, Fla.
248-303-2859

AMERICAN OVERSIGHT

dprieur@wmfe.org
She is available today before 4 p.m. or Wednesday

Danielle saw the *New York Times* story (below) and is considering a short news spot on Zika challenge trials later this week or in the coming months. It appears that she has not done much background research on this topic yet, and I had to explain that the trial discussed in the article has not begun. I let her know that NIAID and WRAIR convened a planning committee in 2016 to determine how to best address ethical issues raised by a possible Zika HCT, and I sent her the resulting report and recommendations. I also sent her Seema's *Science* paper (attached) referenced in the NYT article.

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Ethicists Call for More Scrutiny of 'Human-Challenge' Trials

Global Health
By EMILY BAUMGAERTNER APRIL 20, 2018
A syringe holding an experimental Zika vaccine at the University of Maryland Medical Center. A controversial study in which volunteers are to be infected with Zika has prompted calls for more stringent review of "human-challenge" trials. Credit Noah Scialom for The New York Times
WASHINGTON — Members of a government ethics panel have renewed their criticisms of a controversial study in which volunteers are to be deliberately infected with the Zika virus.
In an article published this month in the journal Science, panel members called for the establishment of ethics committees to review the design of such human-challenge studies, which are sometimes used to test vaccines.
"There is no way to turn back time," said Ms. Seema Shah, a bioethicist at the University of Washington who chaired the panel and is a co-author of the new paper.
"When you're asking someone to take a risk that won't benefit them but may benefit others in the future, you need to know two things — that proper protections are in place, and that it's really going to move the needle."
With funding from the National Institutes of Health, the investigators plan to inoculate participants with potential vaccines and then to inject them with small doses of the Zika virus to test the vaccines' effectiveness. The N.I.H. has not yet decided whether the research will proceed.
The scientists leading the trial say it is necessary to prevent a future epidemic. But Ms. Shah and other bioethicists convened by the N.I.H. concluded in 2017 that the research had "insufficient value" to justify the risks.
People outside the study, such as sexual partners, might also be infected, the panel said. And Zika infection might have unspecified consequences for participants in the long term.
But scientists in charge of the study said the panel's concerns were hypothetical and did not take into account the proposed protocol. They plan to use minimal doses of Zika virus and to quarantine patients in a hospital inpatient unit.
The study also would start by enrolling only women — who are less likely to transmit Zika sexually — and would require them to use long-term birth control.

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"It's really insulting," said Dr. Stephen Whitehead, a vaccine researcher at the N.I.H., said of the panel's decision. "We've been painted as mad scientists who do horrible studies on human beings. But we're on top of all these risks."

Human-challenge studies are only conducted at a small number of institutions in the United States. The trials have been used to test vaccines or treatments for dengue, malaria, influenza and norovirus, among other diseases.

Natural Zika outbreaks are becoming too sporadic to test vaccines, so until the next large outbreak, a human-challenge study may be the only way for vaccine developers to proceed.

"This ethics consultation was debilitating for the whole community," said Dr. Anna Durbin, a research clinician at Johns Hopkins University who collaborated on the study's design. "It really slammed the door on progress."

Jennifer Routh [E]
News and Science Writing Branch
Office of Communications and Government Relations
National Institute of Allergy and Infectious Diseases (NIAID)
NIH/HHS
31 Center Drive Room 7A17C
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AMERICAN OVERSIGHT

From: Pekoc, Ken (NIH/NIAID) [E]
Sent: Mon, 23 Apr 2018 18:54:30 +0000
To: Fauci, Anthony (NIH/NIAID) [E];Folkers, Greg (NIH/NIAID) [E]
Cc: Stover, Kathy (NIH/NIAID) [E];Billet, Courtney (NIH/NIAID) [E]
Subject: (for review) FW: AJC media request on universal flu vaccine

FYI …

From: Oliviero, Helena (CMG-Atlanta) <HOliviero@ajc.com>
Sent: Monday, April 23, 2018 12:47 PM
To: Pekoc, Ken (NIH/NIAID) [E] (b)(6)
Subject: RE: AJC media request on universal flu vaccine

Hi Ken,
Thank you for your help interviewing Dr. Fauci.
I have some good quotes for main story but this is for a sidebar box.
I think it is all OK. Could you give it a quick read over or ask Dr. Fauci to give it a quick read over to make sure I got it right?
If any changes are needed, please let me know.
Deadline is end of today.
Thank you!
Helena

The Universal Flu Vaccine

The AJC recently interviewed NIH's Director of the National Institute of Allergies and Infectious Diseases, Dr. Antony Fauci, who has designating a universal flu vaccine a top priority. Last summer, he brought together more than 150 leading researchers to map a path. Here, he answers key questions about a universal flu vaccine.

What is a universal flu vaccine?

A universal vaccine would offer robust, long-term protection.

One approach to designing a universal vaccine is to generate antibody responses to parts of the virus that are common to all influenza strains that don't readily change during mutation.

Fauci said imagine a head of broccoli with a stalk. The head is the part that mutates each season — and it's also the part the current flu vaccine targets.

A new universal vaccine would go deeper, aiming at the interior "stalk" of the virus, which doesn't change season to season.

Why is a universal flu vaccine needed?

Even an ordinary seasonal flu epidemic will kill some 12,000 to 56,000 people every year in the U.S. alone. That is because seasonal viruses continually evolve and mutate, and although we

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update our vaccines frequently, they may be only 40 to 60 percent effective. This past season, the flu vaccine was only 36 percent effective, and only 25 percent effective against H3N2 influenza, which is causing most flu cases this year.

The goal of the universal flu vaccine would be to offer protection closer to 90 percent for several years with one flu shot.

Where are we in the development and when might we see a universal flu vaccine hit the market?

Several pharmaceutical companies are working on a universal flu vaccine. Researchers at National Institute of Allergies and Infectious Diseases are also working on a universal flu vaccine.

A universal flu vaccine, but only version 1.0, is still at least 5 years a way. A really effective universal flu vaccine that could provide protection against virtually all strains instead of a select few is at least a decade away.

What do you we do while we wait?

About the current effectiveness rate of flu vaccines, Fauci said, "it is absolutely not good enough."

That said, he said, "some protection is better than no protection. Even, 20, 30 or 40 percent protection is better than no protection at all."

HELENA OLIVIERO
Features Writer
The Atlanta Journal-Constitution
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Follow the AJC on Facebook, Twitter or Instagram
Sign up for one of our newsletters

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From: Routh, Jennifer (NIH/NIAID) [E]
Sent: Mon, 23 Apr 2018 18:30:15 +0000
To: Fauci, Anthony (NIH/NIAID) [E];Folkers, Greg (NIH/NIAID) [E];Conrad, Patricia (NIH/NIAID) [E];NIAID OD AM
Cc: NIAID COGCORE;NIAID Media Inquiries
Subject: RE: interview request: Zika HCT/NPR Orlando

O.K. I will direct the reporter to Seema Shah.

Thanks,
Jen

Jennifer Routh [E]
News and Science Writing Branch
Office of Communications and Government Relations
National Institute of Allergy and Infectious Diseases (NIAID)
NIH/HHS
31 Center Drive Room 7A17C
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AMERICAN OVERSIGHT

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By EMILY BAUMGAERTNER APRIL 20, 2018

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"There is no way to turn back time," said Ms. Seema Shah, a bioethicist at the University of Washington who chaired the panel and is a co-author of the new paper.

"When you're asking someone to take a risk that won't benefit them but may benefit others in the future, you need to know two things — that proper protections are in place, and that it's really going to move the needle."

With funding from the National Institutes of Health, the investigators plan to inoculate participants with potential vaccines and then to inject them with small doses of the Zika virus to test the vaccines' effectiveness. The N.I.H. has not yet decided whether the research will proceed.

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But scientists in charge of the study said the panel's concerns were hypothetical and did not take into account the proposed protocol. They plan to use minimal doses of Zika virus and to quarantine patients in a hospital inpatient unit.

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AMERICAN OVERSIGHT

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"This ethics consultation was debilitating for the whole community," said Dr. Anna Durbin, a research clinician at Johns Hopkins University who collaborated on the study's design. "It really slammed the door on progress."

Jennifer Routh [E]
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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Mon, 23 Apr 2018 15:39:24 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Subject: HOLD - AJC interview - Universal flu vax

AMERICAN OVERSIGHT

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Mon, 23 Apr 2018 13:58:11 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Doepel, Laurie (NIH/NIAID) [E];Vance, Meaghan (NIH/NIAID) [C]
Subject: Fwd: AJC media request on universal flu vaccine

Do you want to do this?

Meaghan- can u pls check with ASF.

Sent from my iPhone

Begin forwarded message:

From: "Pekoc, Ken (NIH/NIAID) [E]" (b)(6)
Date: April 20, 2018 at 5:08:49 PM EDT
To: "Conrad, Patricia (NIH/NIAID) [E]" (b)(6) NIAID FOG (b)(6)
Cc: NIAID COGCORE (b)(6) NIAID Media Inquiries (b)(6)
Subject: AJC media request on universal flu vaccine

Hi Patty … would ASF want to do this on Monday?

From: Oliviero, Helena (CMG-Atlanta) [mailto:HOliviero@ajc.com]
Sent: Friday, April 20, 2018 3:54 PM
To: NIAID NEWS (NIH/NIAID) (b)(6)
Subject: AJC media request on universal flu vaccine

Hi there,

I am a features writer for The AJC working on a narrative/explanatory story about this severe flu season and the formidable toll.
I am trying to better understand the development of a universal flu vaccine.
My understanding is a key focus of NIAID's influenza research program is developing a universal flu vaccine, or a vaccine that provides robust, long-lasting protection against multiple subtypes of flu, rather than a select few.

Could you help me better understand how would a universal flu vaccine work?
How would it be different than what is currently on the market?
Where are we in the development? How many companies are currently working on a universal flu vaccine and when we might we see one? in 5 or 7 years. Does the NIAID have researchers working on one as well?
I need this information for a story as well as an explainer box for a universal flu vaccine.

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Thank you in advance for your help.
Hoping to finish by end of Monday.

Helena

HELENA OLIVIERO
Features Writer
The Atlanta Journal-Constitution
223 Perimeter Center Parkway
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holiviero@ajc.com
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Follow the AJC on Facebook, Twitter or Instagram
Sign up for one of our newsletters

AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Mon, 23 Apr 2018 11:27:55 +0000
To: Sonia McKellar
Subject: RE: Re Australian flu 2018

I listed the strains that are covered in my original e-mail to you (see below). Although no intervention is completely without some side effects, the influenza vaccine is considered to be very safe. The most common side effects are soreness at the site of injection and occasional body aches for about 24-36 hours following injection; however, the latter effects are rare.

From: Sonia McKellar (b)(6)
Sent: Sunday, April 22, 2018 9:26 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: RE: Re Australian flu 2018

Dear me fauci, all I know is the (b)(6)
vaccine last year and many did too. (b)(6)
(b)(6)
I hope this year the b strain will be covered in the quad strain.
Can you tell me what strains are covered in the over 65s vaccine ? And what side effects if any are known ?
Regards. Sonia mackellar

On 23 Apr 2018 1:58 am, "Fauci, Anthony (NIH/NIAID) [E]" (b)(6) wrote:
Dear Ms. McKellar:
Thank you for your note. It is important to understand that influenza and parainfluenza are two entirely different viruses. The influenza vaccine that is distributed in Australia, as well as in the USA and elsewhere has multiple components to it: Influenza A H3N2; Influenza A H1N1; and Influenza B. Some forms of the vaccine have 2 different types of Influenza B. Such a vaccine is called "quadrivalent" (4 components). There is no such thing as parainfluenza b. I believe that you might be confusing this with influenza B. Parainfluenza has 4 types: 1, 2, 3, and 4 (no B designation). Also, there are no parainfluenza vaccines. I believe when you were ill in 2017 and your physician took a swab of your nose and mouth that you were actually found to have influenza B. There is an influenza vaccine that is a higher strength that is recommended for people over 65 years old.
I hope that this information is helpful to you.
Best regards,
AS Fauci
Anthony S. Fauci, MD
Director
National Institute of Allergy and Infectious Diseases
Building 31, Room 7A-03
31 Center Drive, MSC 2520
National Institutes of Health
Bethesda, MD 20892-2520
Phone: (b)(6)
FAX: (301) 496-4409
E-mail: (b)(6)

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From: Sonia McKellar (b)(6)
Sent: Sunday, April 22, 2018 7:26 AM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: Re Australian flu 2018

Dear Dr fauci, I have seen your story on 60 minutes and have some questions.

(b)(6)

Can you give me some advice on the new vaccine ?
Thank you.

(b)(6)

AMERICAN OVERSIGHT

From: Eisinger, Robert (NIH/NIAID) [E]
Sent: Sat, 21 Apr 2018 20:52:42 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Doepel, Laurie (NIH/NIAID) [E]
Subject: RE: Lancet Commission final pre-submission draft Review
Attachments: Section 3_041918 rev be ld.docx, Section 4 _041918 rev be ld.docx, Panels_041918 rev be ld.docx

Please find in the attachments the three remaining sections of the Report with Laurie's and my comments.

Thanks
Bob

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Saturday, April 21, 2018 3:00 PM
To: Eisinger, Robert (NIH/NIAID) [E] (b)(6)
Subject: RE: Lancet Commission final pre-submission draft Review

OK. Thanks.

From: Eisinger, Robert (NIH/NIAID) [E]
Sent: Friday, April 20, 2018 6:11 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Cc: Doepel, Laurie (NIH/NIAID) [E] (b)(6)
Subject: FW: Lancet Commission final pre-submission draft Review

Please find in the attachments the:

- Executive Summary (Recommendations/Priorities)
- Section 0 (Report Roadmap/Overview)
- Section 1 (Strategies to reduce TB mortality)
- Section 2 (Research) with modifications from Laurie and me.

Due to the size of the files, I will provide the additional sections (Section 3 – Macroeconomics; Section 4 - Accountability; and Panels – Background on Specific Topics) with our revisions in the next email.

I am on your schedule for Monday morning to discuss the entire document. After our meeting, I will draft an email for you to send to Eric that will highlight the key modifications/issues to the Report.

Thanks
Bob

From: Reid, Michael (b)(6)
Sent: Friday, April 20, 2018 3:14 AM
To: Goosby, Eric (b)(6)

AMERICAN OVERSIGHT

Cc: Dean Jamison (b)(6) Soumya Swaminathan (b)(6)
Subject: Re: Lancet Commission final pre-submission draft Review

Dear Commissioners,

Thank you to all those of you that have contributed to revising the report and responding to the external reviewer comments. In particular, thanks to the teams from NIH and FIND for their help pulling pieces together!

Please find attached a zip file containing the current draft [minus a few pending analyses]. The full report – in 6 sections – can also be downloaded:

From Box: https://ucsf.box.com/s/uqzzpsza4w86x2rmulvyua0bufylpsgi

From Dropbox: https://www.dropbox.com/sh/9se9699agk5s5ck/AAAXQKMeELK728MoCzm-Oifua?dl=0

In order to ensure that we have the best chance of getting the report published in time for the HLM, The Lancet have requested that we formally submit by **April 27th**. I fully appreciate that this means everyone has a short time to review and respond. Nonetheless, we would be grateful for your comments/edits by **April 24** if possible.

In particular, please review the executive summary, in particular the 'vision statement' and the more concise recommendations (5 rather than 10), which align with the HLM 'key asks'.

Eric and I welcome your feedback via email. If possible, share any substantive recommendations/comments in the email, rather than track changes to the documents, to ensure we don't miss any major comments. However, feel free to use track changes if that is your preference. I can also be available most of next week (April 23-26) by 'phone or Skype to discuss questions/clarifications/concerns.

Finally, several of you partnered with non-commissioners to complete writing/analytic products – If you feel these colleagues should be considered as co-authors on the publication, *and you haven't already reached to me to discuss*, please let me know.

Thank you all for your continued support and collaboration in this effort and the important roles you each play in 'building a TB-free world.'

Best regards,

Mike

Lancet Commission on TB, Secretariat

From: "Reid, Michael" (b)(6)
Date: Sunday, April 15, 2018 at 06:22

AMERICAN OVERSIGHT

To: Eric Goosby (b)(6)
Subject: Re: Lancet Commission Conf calls - week of April 16-20

Dear Commissioners,

Apologies for the delay in failing to circle back on this email from David sooner. Unfortunately, we were unable to find any date/time that worked for more than 2 or 3 people. With this in mind, Eric and I have decided to forgo a conference call for all Commissioners.

We are aiming to formally submit a revised draft of the Commission, as well as responses to all the external reviewers' comments, to The Lancet by **April 27**. In advance of that date, I intend to share a pre-submission draft of the full report to all Commissioners this coming Wed/Thurs [**April 18/19**]. I kindly ask that you all take time to review and make edits/comments – sending back any comments/edits no later than April 23.

I can personally be available for one-on-one calls on April 23 and 24 for anyone that wants to give feedback over the 'phone. Please email me individually if you would like to connect on either day.

Thanks for all of your input, particularly those of you that have been re-writing or reviewing subsections of the report over the last 2 weeks.

Kind regards,

Mike Reid
(on behalf of the Commission Secretariat)

From: David McKey (b)(6)
Date: Wednesday, April 4, 2018 at 09:52
To: David McKey (b)(6)
Cc: "Reid, Michael" (b)(6)
Subject: Lancet Commission Conf calls - week of April 16-20

Dear Commissioners,
As a follow-up to our recent meeting in New Delhi, it was agreed that we would arrange for two conference calls the week of April 16-20 to give an update on the progress and discuss any last minute details before the report is submitted the end of April.

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Please find the link for a doodle poll indicating possible times for a call. Please select the two dates and times that work the best for you.
https://doodle.com/poll/nbt5qfuq9x96ddpw

If you plan on submitting expenses for reimbursement, please send as soon as possible. As an FYI, I will be out on holiday 10-22 April. Expenses not received before 6 April will be submitted upon my return to the office.

And, attached is the picture from our meeting.

Thank you,
David

David McKey
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Section 3: Introduction & Recommendations

T~~Everyone dedicated to~~ achieve~~ing~~ an end to TB, affected ~~– impacted~~ countries, donor nations, the private sector, and foundations ~~–~~must redouble their efforts to finance strategies that are working now and, more importantly, strategies that could ~~have the real potential to~~ make a significant impact in the coming years. ~~To end TB, t~~This Commission advocates for substantially more investment in all aspects of TB programming:

- Increase domestic resources ~~mobilization~~ by taxation generation, earmarking, and xx , especially in middle-~~-~~income countries
- Ramp up donor financing to address four priorities: enhancing TB control capacity in low-income countries; reducing the spread, particularly the cross-border spread, of DR TB in all affected low- and middle-income countries~~;~~; and financing the population, policy, and implementation research that will yield ~~provide~~ lessons ~~learned~~ learned and international sharing of best practices.

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3.0 Macro-economics of TB

3.0.1 The basics of TB economics

3.0.2 Costs per death averted

3.0.3 The economic burden of tuberculosis

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LANCET COMMISSION ON TUBERCULOSIS: SECTION 3

3.1 Domestic Financing for TB

The section above makes the case for economic benefits of investing in TB R&D. In this section, ~~of the report,~~ we discuss the critical shift in the last two decades from international to domestic financing for TB. We examine the extent to which TB currently relies on domestic sources of finance; and the influence of domestic financing on the sustainability, efficiency, and equity of TB program funding. In addition, we explore the potential for rapidly increasing domestic financinge for TB programs in the coming five years. Finally, we highlight the importance of investing in the systems that allocate, distribute, and manage domestic TB resources: ~~i~~It is essential that such systems are developed if increased financing is to be spent effectively and efficiently to end the epidemic.

3.1.1 The pivotal role of sustained domestic financing for TB

Improved domestic financing for TB is one of the success stories in global health in the past two decades. In~~By~~ 2017, 84% of global funding for TB came from domestic sources. This high proportion reflects a consistent pattern of increased commitment to TB from high-burden countries. From 2007 to 2017, global funding for TB doubled, with much of the increase coming from Brazil, Russia, India, China, and South Africa (BRICS). On average, the BRICS nations have domestically financed 95% of their public TB expenditures over the past decade [1].

Outside of the BRICS countries, the picture of domestic funding for TB is complex, reflecting a general scarcity in health sector resourcing and capacity. In 2017, less than half of public funding for TB in low-income countries came from domestic sources. However, the trend over time is promising; on average, low-income countries doubled their domestic financing of TB between 2007 and 2017, with a rate of increase similar to that of international TB funding to their countries[2]. Not all low-income countries are following this trend~~;~~, so there is room for improvement;~~,~~ ~~with~~ the current proportion of the domestic contribution to public TB expenditure rangesing from under 1% to 24%[2]. Likewise, in lower-middle income countries, the proportion of domestic public funding ranges from 7% to 88%[2], with the average growth in domestic TB financing stable until 2013, but doubling since then.

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3.1.2 Who provides domestic finance, and how does it flow to TB?

TB expenditures can be divided into those that flow through general health service provision and those that flow through National Tuberculosis Programmes (NTPs). While domestic contributions to total public TB expenditures generally are high, NTPs and TB-specific commodities are more reliant on international finance. In 23 of the 30 high-burden countries, NTPs receive more than 80% of their funding externally[2], with the Global Fund being a substantial payer of TB commodities. This apparent financial dependency of NTPs on international finance for TB commodities has come about because disease-focused international funds lack flexibility compared ~~to~~with domestic funds. It does not reflect an overall lack of domestic commitment to TB.

Domestic financing for TB within countries can come from a range of sources. Ultimately it is populations and firms who pay, but ~~TB patients still face much of the burden in some countries.~~ ~~D~~despite the policy of free or reimbursed TB care in most countries, patients with TB can still incur substantial out-of-pocket payments for public TB services [3]. Moreover, in several high-burden countries, large proportions of patients seek and receive TB care in the private sector, paying for their own care and treatment. In line with the goal of Universal Health Coverage (UHC), subsidizing and pooling these private domestic expenditures will have beneficial consequences in terms of financial risk protection[4,5] and possibly health outcomes[6] for those with TB.

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3.1.3 Is the allocation of domestic finance to TB efficient?

Although many countries have increased their allocation of public monies to TB, a mismatch remains between allocation and need, the latter defined in terms of the resources required to reach global End TB targets [2]. From a domestic public finance perspective, however, need is not a sufficient criterion to increase investment. Ministries of Finance will have requests to fund many other development and health interventions that have potentially high returns. Hence, those advocating for increased investment in TB, both within and external to governments, need to demonstrate that investment in TB performs well, at the very least compared ~~to~~with other health sector investments.

Increasingly countries are developing public finance processes that formally assess the return to investment of different health sector interventions, rather than relying on global evidence. These

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processes are being supported by improved data and an understanding of the costs, effectiveness, and long-term impacts of investment in TB on both health and economic outcomes[7]. In the main, supporting these efforts often works in favor of TB. In Malawi, for example, a recent assessment to determine the essential package of health care found that 7 of the top 10 investments were TB interventions [8]. This mirrors systematic reviews of return to investment of TB expenditures across several countries [9]. Therefore, in many countries, the case for increased domestic allocation to TB includes its role in improving the efficiency of the entire health sector.

~~There is, h~~However, ~~room to improve~~ the efficiency of TB expenditures can be improved, through improvements in both the delivery and implementation of TB services, as highlighted in Section 1. In some countries the split of TB expenditures on TB commodities versus general health service provision may not be optimal. Improvements in health system strengthening are critical to ensuring that health staff at the front end of TB service delivery receive the right mix of resources to provide high-quality, patient-centered TB services [10]. Some countries also have higher-than-average TB treatment costs, due to the over-hospitalization of TB patients. The decentralization of DR TB care in South Africa illustrates the substantial additional funding that may be generated by reducing hospitalization for certain patients [11] . Improved integration of TB services may also support patient-centered care and reduce costs [12]. Several new TB technologies, such as shortened regimens, may reduce the costs substantially. Unfortunately, there remains a dearth of information on the efficiency of different approaches to scaling up TB services to guide how countries can spend funding effectively [13].

3.1.4 Can domestic funding for TB be substantially increased in the next five years?

Generating additional domestic financing for TB depends on several factors: the government's commitment to allocate more funding to TB; the future potential for efficiency gains; and increases in the overall level of available public finance. Increases in domestic financing for TB in the past two decades demonstrate that countries experiencing GDP growth may be able to expand their funding of TB rapidly, and at the same time reduce TB incidence[14]. In addition, the ability to raise domestic finance for TB from private individuals and firms depends on the system of revenue generation and taxation structures. In recent years, a range of innovative mechanisms, including earmarked taxation of alcohol and cigarettes, the expansion of health insurance coverage, and social impact bonds, have all been

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explored to improve the financial sustainability of the health sector, with positive consequences for population health [15].

~~Conducted i~~In collaboration with the Lancet Commission, a team at the London School of Hygiene & Tropical Medicine (LSHTM) and UCSF conducted an analysis examining the potential fiscal space for TB in the 30 high-burden countries (HBCs) over the next five years. Fiscal space analyses apply international public financing norms to current fiscal performance to determine the extent to which funding can grow in a way that does not damage overall fiscal stability. The financing sources examined included GDP growth, increasing public revenues, improving allocation to the health sector, improving allocations to TB, and increasing the efficiency of public TB service delivery. The researchers found that most high-burden TB countries can substantially increase public domestic financing of TB. By 2023, countries such as Bangladesh, Zambia, China, and Indonesia can potentially increase their annual TB expenditures more than five-fold. Economic growth and especially improved taxation will be pivotal in some countries, but on average will generate modest increases in TB funding. The largest increases would come from improved resourcing of the health sector, ~~either~~ through increasing allocation of public resources to health, pooling financing, and earmarking tobacco taxes to health. Commitments by Ministries of Health ~~in~~to prioritize~~ing~~ TB within the health sector and improve~~ing~~ service efficiency will also be important, particularly in some low-income countries like Zambia and Tanzania. (Figure 3.2.1)

Figure 3.2.1

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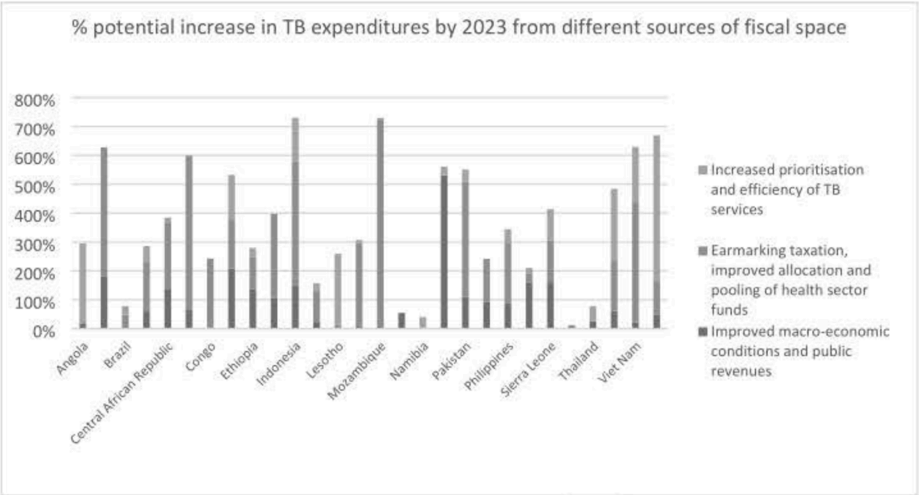

3.1.5 Policy Implications

In summary, mobilizing domestic resources for TB, will take policy action and commitment across government, from politicians, Ministries of Finance, and Ministries of Health. Increasing tobacco taxation is a clear policy action that can support financing TB elimination and have positive benefits for persons with TB. Supporting UHC will be pivotal to sustaining domestic financing for TB in a manner that protects TB patients from catastrophic expenditures. As such, ending TB is contingent on the success of the UHC agenda.

However, it should not be assumed that commitment to this broad policy agenda is sufficient. Rapid increases in domestic financing for TB will require enhanced capacity to allocate and spend resources effectively, transparently, and accountably, and to demonstrate results. The lack of such 'absorption' capacity can fundamentally constrain the rate at which additional financing is disbursed in practice, once committed. In addition, rapid disbursement will depend on the capacity of NTPS to mobilise expertise, infrastructure, and sufficient human resources in a timely manner. Upfront support to NTPS to build the mechanisms to absorb new funding, and the effective support to resource allocation and management

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systems and processes within the health sector, will therefore be critical to ensure additional resources are used. The commitment of many HBCs over the past two decades is commendable, and many have the space and willingness to do more, but achieving real increases in expenditures~~,~~ beyond the current rate will require concerted attention by all those working to end TB to absorb additional resources effectively.

3.2 Donor Financing for TB

3.2.1 Donor investments in TB

The potential for increased domestic health spending, economic growth~~,~~ (as mentioned in Section 3.1), ~~along with~~and the recent rise of populism and protectionism~~,~~[16] ~~will~~ inevitably will shape financing for TB programmes over the coming decade. Nearly all high-burden countries can substantially increase domestic resources allocated to TB. While many low-income countries still require donor financing for TB, new opportunities exist to re-think how and where donor financing is allocated to maximize its impact.~~such that its impact is maximal.~~ In this section ~~of the report~~, we discuss the role of donor financing to catalyze domestic efforts and invest in global public goods, especially in those countries transitioning out of donor finance eligibility. In addition, we highlight the potential benefits to donor partners of investing in TB, economically and in terms of addressing the negative cross-border externalities that TB, especially DR TB, poses. Finally, we underscore the importance of sustained financing for the poorest countries and advocate for continued investment to end the epidemic in those countries.

3.2.1 Who is investing in TB programs?

According to the Organization for Economic Co-Operation and Development's C~~c~~reditor R~~r~~eporting S~~s~~ystem, international donors provided US$754 million for TB prevention, diagnosis, and treatment in 2015; 64% of this was provided by the Global Fund, of which the United States (US) was the major contributor.[17] In addition, the US supported a further US$199 million channelled via its own agencies and other institutions. Overall, approximately 47% of international donor funding originated in the US in 2015. Aside from Global Fund and the US government, remaining funding came from the United Kingdom (3%), the World Bank (1%), and other sources (6%). While the Global Fund has consistently

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been the largest provider of international donor TB funding over the last decade, current levels of funding are far below a peak of US$712 million in 2013. Furthermore, overall funding for TB still falls very far short of the annual US$2.6 billion proposed in the Global Plan to End TB, outlined by the Stop TB partnership.[18] ~~Donor financing for TB has been dwarfed by HIV and malaria funding over the last decade.~~ The Institute for Health Metrics and Evaluation (IHME) projects that in 2016, development assistance for health (DAH) for TB reached $1.4 billion, or just 4% of total DAH (Figure 3.2.1)[19], in spite of TB being the leading infectious disease cause of death globally.

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3.2.2 How is donor finance being used?

Analyses of donor financing for health have traditionally tracked flows by funding source, channel, recipient, and disease. For this Commission, a team at SEEK, Duke University, RTI International, and UCSF has conducted an analysis of DAH for TB broken down into functions (Annex 3.2.1).[20] The researchers analyzed overseas development assistance (ODA) for TB in the year 2015, the most recent year for which data are available in the Creditor Reporting System, which provides detailed information on aid activities.[21]They found that in 2015, there was US$932 million invested in ODA invested in TB control (estimates by IHME, the SEEK/Duke/RTI/UCSF collaboration, and by the WHO of the annual amount of DAH ~~are all~~ differe~~nt,~~ because they use different methodological approaches; see Annex 3.2.2.). Of this ODA for TB, 53% went to lower MICs, 20% to LICs, 4% to upper MICs, and the rest was unspecified. Only about one-fifth (22%) of ODA for TB was for global functions, broken down as shown in Figure 3.2.1, and around four-fifths (78%) was for country-specific functions. Almost all (over 99%) of the country-specific support was for TB program funding, with less than 1% directed at system-wide, cross-cutting health systems strengthening.

3.2.3 Policy implications

To our knowledge the analysis outlined above is the first to determine how much TB-specific aid is devoted to supporting global functions versus country-specific functions. Acknowledging the limitations—~~-~~that this analysis sheds no light on trends in funding or how country-specific TB program funding is disaggregated between DR-TB and DS-TB control efforts—~~-~~these findings have three important policy implications, which are reviewed below.

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LANCET COMMISSION ON TUBERCULOSIS: SECTION 3

1. **Increased investment is needed to support Global TB functions.** The first implication is that funding for TB-specific global functions should be strengthened (Table 3.2.1). Although our baseline analysis cannot prove that global functions are being neglected, it does provide suggestive evidence. This Commission asserts that donor financing should increasingly be focused on the following global functions:

Supplying global public goods ~~(GPG)~~ - Greater investment in ~~-~~global public goods (GPG), in particular TB R&D and intellectual property for new drugs and technologies, is likely to bring important economic benefits and have a disproportionately beneficial impact on health outcomes in low- and middle-income countries (LMICs).[22] New tools deriving from TB R&D are also likely to provide financial protection and be pro-poor, as shown by "extended" cost-effective analyses.[5] Another GPG, and an existing Global Fund priority, is funding market-shaping activities (such as pooled procurement to reduce second-line drug or molecular diagnostic pricing). This will remain important not only for ~~low-income countrie~~LICs but also for ~~as~~ countries transitioning out of donor funding eligibility, as they ~~and~~ would otherwise be unable to access concessional prices for such drugs or diagnostics.

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Exercising leadership and advocacy- An important, albeit often neglected global function of aid relates to investment in health advocacy and priority setting. This includes, but is not limited to, ~~to~~ donor financing to support the role of civil society in TB control efforts. Recognizing that funding for HIV advocates and activists has been crucial to global HIV efforts,[23] this commission affirms the importance of increased funding for TB advocates as a global public good, deserving investment commensurate with the part it plays in improving health outcomes. Increased funding to support the roles of ~~the~~ WHO should also increase, to facilitate uptake of new policies, strengthen surveillance systems, and be a conduit for technical assistance.

2. **Targeted investment is needed for countries graduating from ODA -** Presently, 60% of country-specific aid in our analysis is directed towards middle-income countries, many of which will soon be ineligible for donor financing; based on their national GDP per capita, they are becoming 'too rich' to qualify for DAH. Unfortunately, many of these countries are likely to have large pockets of poverty and avertable mortality from TB. Here we propose targeted investments focused on high-risk populations and/or to enable optimization of domestic resources towards TB control. Furthermore, we argue that sustained funding in many of these countries, especially those with a significant DR TB burden, is warranted given the global implications of failing to ensure TB control in these settings.~~:~~

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Protecting risk pools – Prisoners, people living with TB/HIV coinfection, migrants, refugees, and indigenous populations are all highly vulnerable to TB, and they experience significant marginalization, decreased access to quality services, and human rights violations. These communities will continue to benefit from donor support, even as domestic resources for health are increasing, not least because they are often last in line to receive domestic resources.

Management of cross-border externalities – As highlighted in Section 1 ~~of this report~~, the high cost of treatment for DR TB, especially in middle-income countries[3], has been a significant barrier to scaling up treatment provision to date, and it will continue to rise over the coming years.[24] Donor partners, especially the Global Fund, are already investing disproportionately in DR TB control activities. Nonetheless, given the substantial weight of data demonstrating extensive cross-border spread of DR-TB, [25-35] as illustrated in Figure 3.2.2, DR TB poses perplexing economic and health security issues for donor countries, even those pursuing increasingly isolationist foreign policy agendas. It is important that sustained funding for DR TB control efforts, even in countries that will be soon 'graduating' out of ODA eligibility, be sustained to mitigate the cross-border threat that DR TB poses. Aligning DR TB control efforts with the broader AMR agenda is also essential to maximize investment; unchecked, TB will be the single biggest cause of antimicrobial resistance-related deaths by 2050.[36]

Co-financing and catalytic funding - In addition to *where* DAH is spent, *how* it is spent is also crucial to guaranteeing the impact of donor support. Catalytic investments, such as those supported by the Global Fund, offer examples of how new models of financing, through use of matching funds to incentivize country allocation for priority areas, or multicounty funding mechanisms that address specific priority areas. Notwithstanding the need for better data assessing the impact of these funding mechanisms, co-financing solutions provide an important pathway to ensure greater country ownership while also ensuring sustained funding for TB activities even during the transition process.

3. **Ongoing support is needed to support the poorest countries.** By 2035, there are still likely to be approximately~~round~~ two dozen ~~low-income countrie~~LICs that will require direct country assistance for years to come.[4] Donor financing for these countries needs to increase substantially to make up for funding shortfalls over the last few years; although the funding for TB increased 13.3% from 2015, it still fell *very far* short of the annual $2.6B in DAH that is needed for TB according to the Global Plan.[17] The moral imperative of sustained donor investment in these countries should be highlighted—~~—~~millions of individuals will potentially die from TB in these countries without external

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assistance. In addition, the scale of the impact of those avoidable deaths on the global economy is substantial, as our analysis in section 3.0~~,~~ highlights. Investing in TB control will reap economic dividends that will likely benefit both donor and recipient nations. Underscoring the importance of investing in TB as an important tracer for progress towards UHC[37] should also inform how and where donor funds are allocated. As global momentum builds towards achieving UHC, investment in TB as disease of poverty is imperative to that progress.

3.2.4 A new era of shared responsibility

As highlighted at the HLM on TB, priorities and approaches to financing TB are evolving; we are entering a new era of increased country ownership and global cooperation.[16,38] In addition, the architecture of donor financing for TB is changing as high-burden countries mobilize additional resources for TB control. Leveraging concessionary loans from development banks[39] and innovative financing mechanisms (e.g. social impact bonds, loan guarantees)[16,18] may play an increased role. Even in LIC~~low-income countries,~~ still reliant on donor support, the nature of donor-recipient financing must evolve. Partnership agreements between donors and recipients, as a tool to ensure ownership, accountability, and transparency, should be encouraged. New models of donor financing~~,~~ that focus on results, encourage innovation, and strengthen government accountability to citizens rather than donors are also necessary. One promising example of a new financing strategy, Cash on Demand Aid, whereby donors pay for measurable and verifiable progress on specific outcomes, could be deployed to improve TB quality or private sector engagement efforts.[40] In addition to new funding mechanisms, new funding partners, such as multinational business and corporate philanthropists, should be encouraged to close TB funding gaps. The opportunity for legacy impacts at national and global level, an oft-cited motivator of such funders, will increase as TB elimination efforts become tangible.

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Table 3.0.1 Implicit estimates of cost per TB death averted

Source	Description	Cost per death averted (US $)
Global Health 2035[1]	Estimated total cost per year and TB deaths averted in 2035	
	(i) Low-income countries	$5,400
	(ii) Lower-middle income countries	$6,000
Stop TB (Global Plan to End TB)[2]	"Standard investment scenario" (all but OECD countries, 2016-2020)	$6800[a]
WHO Global TB Report[3]	The document provides estimates	
	(i) Drug sensitive TB	$7,500
	(ii) M/XDR TB	$16,000

OECD=Organization for Economic Cooperation and Development

[a]Calculated assuming 1 death averted per 25 DALYs averted

[b]Cost per death averted =$25 \times cost/DALY$. Alternatively, $cost/death\ averted = \frac{cost/case\ treated}{(treatment\ success \times CFR) \times [1+\alpha]}$, where a is a transmission effect parameter, assumed here to be zero.

Sources: 1. Jamison et al (2014); 2. STOP TB (2015); 3. WHO (2017)

Note: These estimates are implicit in the sense that they are not provided in the source but instead calculated from information in the source.

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Table 3.0.2 Total value of mortality gains: 90% of tuberculosis deaths eliminated in 2015

		Population (in thousands)	TB deaths (in thousands)	Total Value (in billions)	Total Value as % GDP	Total Value per Capita
VSMU conditional on age	Low	1,288,244	272	-$9.73	-1.41%	-$7.55
	Lower-middle	4,126,608	784	-$117.44	-1.98%	-$28.46
	Upper-middle	2,802,574	97	-$64.29	-0.32%	-$22.94
	High	1,282,990	27	-$110.00	-0.21%	-$85.74
	World	9,504,210	1,210	-$1,366.70	-1.72%	-$143.80
VSMU unconditional on age	Low	1,288,244	272	-$13.29	-1.93%	-$10.32
	Lower-middle	4,126,608	784	-$159.16	-2.68%	-$38.57
	Upper-middle	2,802,574	97	-$93.42	-0.47%	-$33.33
	High	1,282,990	27	-$160.43	-0.30%	-$125.04
	World	9,504,210	1,210	-$1,902.18	-2.39%	-$200.14

Note: Assumed 2045 TB death rate that of 2015 and that 90% of such deaths were reduced.

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Table 3.2.1. The global and country-specific functions of DAH for global TB control

Function of DAH	Examples as applied to TB control
Global functions	
Providing global public goods (GPGs)	▪ Population, policy, and implementation research on preventing and treating TB ▪ Development of TB medicines, vaccines, and diagnostics ▪ Generation and global sharing of knowledge on TB control strategies ▪ Global system of financial monitoring of TB control[1] ▪ Global pooled procurement of TB drugs[2] ▪ Regional and global health technology assessment for TB technologies
Managing regional and global negative cross-border externalities	▪ Tackling antimicrobial drug resistance ▪ Controlling the cross-border regional and global spread of TB and MDR-TB
Supporting global leadership/stewardship	▪ Global priority setting for TB control ▪ Convening of TB policymakers for negotiation and consensus building ▪ Promotion of the effectiveness, value for money, and accountability of external financing (DAH) for TB
Country-specific functions	▪ Direct financing and technical assistance to a country for TB national control activities ▪ Support for national health systems strengthening

Source: 1. Floyd K et al (2007), 2. Arinaminpathy N et al (2013)

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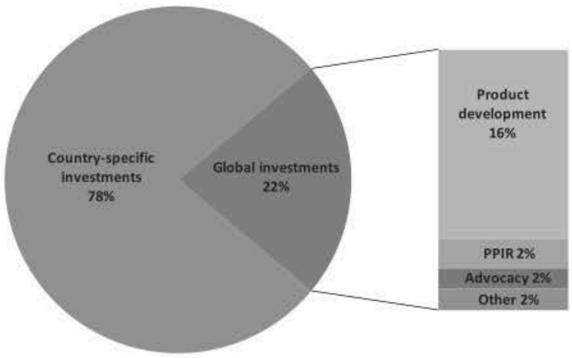
Figure 3.2.1 Breakdown of 2015 ODA for TB by function



PPIR: population, policy and implementation research.

Figure 3.2.2

Cross-border spread of drug-resistant tuberculosis (tracked by M. tuberculosis genotype)

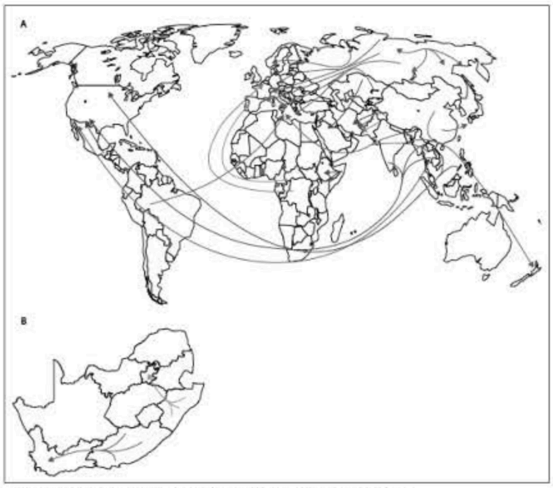


Figure 3: Inter-country and intra-country spread of drug-resistant tuberculosis according to *M tuberculosis* genotype
(A) Worldwide spread of drug-resistant strains of *M tuberculosis*. Red=Beijing strain,[90,96,98,99] Green=LAM 9 strain,[100] Light blue=Haarlem1 strain,[104] Purple=T1 strain,[106] Dark blue=untyped strains.[106,108] (B) Ongoing intra-country spread of extensively drug-resistant tuberculosis strains in South Africa. Red=atypical Beijing strains. Green=LAM4 strain.[110,109] Intra-country spread has also been reported in Portugal[107] and Spain.[108]

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465 Source: Dheda, K et al, The epidemiology, pathogenesis, transmission, diagnosis and management of
466 multidrug resistant, extensively drug-resistant and incurable tuberculosis. Lancet Respir Med, 2017

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Lancet Commission on Tuberculosis

Section 4

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4.0 Introduction & Recommendations

In ~~s~~Section 4 we highlight the importance of an enabling environment in each country's response to TB. Figure 4.1 provides a framework to reflect on and operationalize country-owned responses that will drive progress towards ending TB ~~as a public health threat~~ and leverage good practices in the TB response to advance other Sustainable Development Goals. This framework sets out an idealized response that illustrates mutually reinforcing functions performed by state and global actors. These functions are person-centered, rights-based, data-informed values. A strong TB response needs to be guided by country-owned, multi-sector and multi-stakeholder coordination and accountability. Civil society is a crucial constituency that can ensure accountability for TB programs and TB stakeholders at ~~both~~ global, national, and subnational levels. This framework also underscores the importance of addressing TB as part of achieving UHC. While countries are in varying stages of progress towards UHC, prioritizing investments in TB to realize UHC will be important in high-burden countries. UHC, backed by donor assistance, also offers an opportunity to tackle TB with multisectoral initiatives as predicated consistent with the principles of the Sustainable Development Goals.

This Commission recommends:

- Accelerate progress towards UHC; strong national TB programs that can prioritize specific TB care and prevention functions within a progressive universal pathway to UHC are essential in high-burden countries.
- Fortify the leadership and engagement of civil society in all aspects of TB programming, strengthening and increasing their decision-making roles in policy, implementation, and accountability, investing in their involvement for their global public good.
- Establish multi-sectoral accountability mechanisms, including the creation of a country report card, to ensure that progress is being achieved across the four domains identified above.

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4.1 Ending TB is important on pathway to achieving UHC

As this report highlights, progress towards ending TB ideally will occur hand-in-hand with achieving UHC. UHC means all people have access to high-quality health services—at a minimum, health promotion and primary care—at no or little cost at the point of service. This Commission asserts that ending the TB epidemic must involve strong national TB programs that can prioritize specific TB care and prevention

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functions within a progressive universalist pathway to UHC.[1] As countries design UHC programs, TB care and prevention functions should be addressed specifically and contained within essential service packages.[2,3] Progressive social insurance models that prioritize diseases that disproportionately affect low-income and other vulnerable populations will automatically incorporate TB. To realize the End TB targets, this Commission proposes to reach populations at highest risk for TB early in the roll-out of such schemes. In countries with high TB burdens, maintaining a separate TB budget and program within a broader UHC framework typically will prove efficient. However, as the TB burden declines, integrating the TB programs into primary health care platforms will become desirable.

Several other system-wide frameworks are relevant to a TB-inclusive UHC agenda. These include ensuring the uninterrupted availability of and access to appropriately regulated TB medications and diagnostics, strong information and performance systems, and new or merged risk financing pools.[4] Regulation should address how medical products are subsidized as well as the types of medical professionals authorized to prescribe or dispense TB medicines. High-burden countries will also need to establish an optimal mix of skilled health workers to deliver services, and to design appropriate pay incentives for health professionals to support scaling up the TB response as well as a broader UHC agenda.[5]

4.2 Social pProtection

The adverse financial consequences of TB on households can be profound and long-lasting, as illustrated in Panel 4.2. To reduce these requires implementing policies that can protect patients and their households against ruinous financial costs associated with TB. Especially in those settings where private sector care predominates, strategies must be deployed to achieve financial protection and adequate quality of care when private providers are used. This Commission argues that, as part of the UHC agenda, public finance should be extended to private providers for TB care, and that private finance in public facilities (user fees) should be minimized. Beyond public financing of treatment and case-finding, many TB patients also may need ~~also~~ economic and social support. These measures, particularly, social support, can enhance treatment adherence and positively affect clinical outcomes.[6]

Social protection interventions—policies and programs designed to protect individuals from social and economic risk while improving social development,[7]—are a promising approach to improving TB

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outcomes[8,9] and achieving these larger policy goals. Examples include cash transfers and nutrition programs offered as part of national policies. Such interventions can contribute to successful TB outcomes indirectly by addressing social, biological, and structural determinants, or contribute directly by enabling access to care.[10-12] Recent modelling work leveraging data from 192 countries to link the SDG targets to TB outcomes projects that expanding social protection schemes to all eligible populations could profoundly impact the epidemic.[13]

4.3 Sustaining top-level political support and leadership

Strong national and local political leadership creates an environment conducive to sustained attention and funding. To end TB, governments of high-burden countries will need to propose bold plans to end TB rather than be content with modest incremental gains. Encouragingly, there is growing political recognition that countries need to act now to address the TB epidemic. Since its establishment in 2014, the Global TB Caucus,[14] which supports 2,300 parliamentarians in 130 countries, has become a driving force to mobilize political capital to address TB. Examples of TB legislation in the Philippines[15] and Peru,[16] mobilizing national finances to drive improvements in TB care and prevention, highlight successes achieved because political leaders in these countries championed the cause. In South Africa, political leaders from Ministries of Health and Finance have been instrumental in formulating a TB investment case to mobilize additional resources to find new cases and treat more drug-resistant TB (Panel 4.3). Progress as dramatic as that envisioned in the End TB strategy can be achieved only when each country's leadership outlines a long-term strategy to fight TB within its borders, similar to longstanding strategies established to fight HIV/AIDS.

Effective leadership at the National Tuberculosis Programme (NTP) level is also a critical element of a successful TB response and evidence of high-level commitment to addressing TB. The size and capacity of the NTP's central coordination team and the level of decentralization and integration of specific services depend on many factors, including the country's size, governance, administrative structure, and TB epidemiology. However, chronic underinvestment in TB control efforts can undermine all aspects of TB programming, including the caliber of NTP key personnel, human resource planning, capacity strengthening, and supervision and monitoring of service quality. Empowering NTP managers to take the necessary steps to institute effective strategies will require increased financing and recognition that NTP leaders must play an inter-sectoral, convening role with stakeholders of other government ministries,

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including finance, justice, labor, social welfare, housing, mining, and agriculture. To ensure the high-caliber NTP leadership needed to drive substantive change demands that these managers receive adequate pay, reasonable autonomy, and opportunities to maintain up-to-date technical knowledge.

4.4 Maintaining multi-sectoral engagement

In the SDG era, addressing TB must occur as part of a broader multisectoral framework that addresses key social determinants—especially poverty,[17] undernutrition[18], and smoking[19]—clearly linked with TB and TB mortality. To achieve this will require collaboration among multiple ministries, agencies, and civil society. The health sector, particularly the NTP, can play a key role in identifying and communicating the potential health impact of policies on food security, improved housing, poverty reduction, employment safeguards, and human rights protections for migrants, prisoners, and other marginalized groups.[5] Numerous policy tools, including taxes and subsidies, laws and regulations, information and communication, and improvements in the built environment, should be employed to address these issues. As highlighted below, national accountability to address these determinants, at both athe national and subnational levels may be valuable, especially in addressing issues such as tobacco control and under-nutrition.

Commented [ER([2]: What does this mean?

While not disavowing the critical importance of a multisectoral agenda to address determinants of TB disease, this Commission recommends that improving access to diagnostic, treatment, and preventive services, especially for high-risk populations, should be the primary means of ending TB as a disease of global public health significance, in most high-burden countries. Over the next generation, substantial progress can be made by ensuring that individuals with TB can access curative treatment, and that those at highest risk for TB disease can access to preventive therapy, especially becausesince so many at-risk individuals lack that access currently. Continued improvements in TB control tools and the systems for delivering TB programs coupled with greater financial resource mobilization for health offer the most concrete likelihood of ending the epidemic.[20]

4.5 Strengthen civil society involvement in all aspects of the TB response

A critical lesson learned from HIV/AIDS response is that engaging stakeholders from civil, public, and private sectors requires national leadership to bring disparate actors together, overcome barriers, enable policies, and scale up access to effective medical tools. Civil society dramatically changed the global response to HIV/AIDS, making it a top priority at all levels and driving unprecedented growth of

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donor support for lifesaving interventions.[21] Such a response to TB will also require partners to commit to broad outcomes rather than just pursuing their niche goals .[22] In addition, TB-affected communities must be empowered to participate in shaping policies and programs. Despite recognition from key global stakeholders that TB-affected communities must be involved in decision-making bodies, the gap for greater involvement remains huge. The mantra of the disability movement— 'nothing about us without us'— could not be more relevant.[23]

Until recently, few TB survivors or other people affected by the disease have served as public advocates, partly because of TB's curable nature, the vertical top-down orientation of TB control efforts, the persistent stigma of TB worldwide, and the lack of funding to support community involvement in TB programming.[24,25] Fortunately, this is changing. A growing cadre of healthcare workers and students who are TB survivors are using their dual perspectives and professional networks as platforms to call for rights-based services and accelerated access to diagnostics, new treatment regimens, and vaccines.[24] National and transnational TB activism is emerging as a vital force advocating for services in hard-to-reach populations, mobilizing communities and strengthening community systems. TB survivors can play an essential role in creating incentives for political leaders to make difficult and risky decisions, in generating public support for those decisions, and in holding leaders and service providers accountable for how resources, commitments, and services are delivered. In the post HLM-era, the continued input of TB-affected civil actors is essential to ensure the accountability of politicians and program planners. Recognizing their contribution as a global public good, governments and international organizations must create conditions for civil society actors to play an expanded role in the fight against TB, supporting their contribution through direct investments and assembly to raise inconvenient truths. This should include involving such advocates in national TB strategic planning processes, national TB research-agenda setting activities, and national and regional accountability mechanisms. In addition, donors and governments need to recognize the importance of funding civil society groups to provide that contribution.

4.7 Ensuring a hHuman-rRights aApproach to TB

A crucial lesson from the HIV epidemic (and for global health generally) is that only by the committingment expressed toin universal human rights for to enjoyment by everyone canforof the highest available standard of physical and mental health care can be fulfilled.[22] To uphold and defend

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the human rights of people with TB or those at most risk of TB can bring down rates of infection and death. Practical solutions are needed to expedite changes in the laws, policies, and public attitudes that violate human rights of vulnerable populations who might be at particular risk of developing TB disease, including people living with HIV, prisoners, refugees and migrants, miners, and health care workers. Furthermore, human rights must be ~~an~~ integral ~~dimension in~~to the design, implementation, and evaluation of an integrated and multisectoral response to TB.[26] A human rights approach to TB research is necessary to ensure that ~~there are~~ legislative and policy frameworks exist~~in place~~ to ~~ensure that benefits of scientific progress are available – to~~ enable the diffusion of encouraging new scientific discoveries, provide accountability for R&D investments[27], and ~~through~~ remov~~e~~ing barriers that preclude new TB research technologies being broadly available for public benefit. [28]

4.8 WHO – a new role for new era

With greater emphasis on domestic resources and the centrality of national health systems, the SDG era offers a unique opportunity to better define the role of WHO in ending the TB epidemic. This Commission identifies several priorities where the WHO can be an important catalyst for change. First, while technical assistance to countries and strategic leadership may not be unique to WHO, ensuring their adequate and neutral provision to member states is crucial.[29] Second, the WHO global TB program must catalyze rethinking and modernizing surveillance systems and data analytic platforms.[30] WHO has a crucial role to play in supporting countries to leverage these systems to improve the quality and efficiency of NTPs~~TB programs~~, enable greater accountability, and facilitate more responsive and targeted technical assistance. Third, WHO should expand the reach and quality of its advocacy and strategic communication capacity to ensure that global guidelines are clearly understood and implemented by all relevant constituenci~~audienc~~es.[29] Finally, together with the World Bank and development partners, WHO should advocate for increasing Development Assistance for Health (DAH) for TB for low-income countries and for control of DR TB. It can and should play a more direct role in advising how that money is targeted and prepare TB programs for successful graduation from DAH through hybrid mechanisms like the Global Fund and the Global Financing Facility.[31]

~~The~~ WHO's Director-General has repeatedly asserted the importance of UHC to his tenure,[32] committing to 'making universal health coverage happen in our lifetime.'[33] Accordingly, WHO must continue to support strong vertical TB programs as a central tenet of UHC. To end TB needs both a focused

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commitment to TB activities and a progressive, inclusive vision of health care. WHO has a crucial role in supporting countries to hold these two complementary priorities in tension.

4.9 Establishing local, national, and global accountability

Turning written commitments into substantive actions will require an accountability framework that tracks all elements of the TB response occurring at global, national, and local levels. This framework needs to measure progress towards ending TB globally and include timely reviews of results through government and civil society accountability mechanisms at national and global levels. It also must incorporate a means for taking appropriate corrective actions.[34]

At a national level, this Commission proposes a framework to ensure accountability that extends beyond national TB programs and reports directly to heads of state. The reasons that TB should, as an exception, report to heads of state include the health security risk it poses, and its adverse impact on national economies and health systems. Such a framework should include specific targets for reducing mortality and detecting more cases, screening high-risk populations and scaling up access to preventive therapy, consistent with national strategic plans, as well as addressing inequities in TB risk across populations. In addition to tracking epidemiologic metrics, it must also track domestic investment in TB control. Furthermore, it should engage ministers across government to ensure multisectoral accountability on issues such as tobacco taxation and the regulation of air pollution. National TB Commissions or cabinets that can monitor progress across sectors and/or ensure implementation of TB-specific national strategic plans may be appropriate in high-burden countries. Enabling subnational accountability, leveraging regional data to highlight gaps in services and opportunities for allocative efficiency, is also likely to be effective. Linking accountability mechanisms to financial resources that are allocated separately from health budgets, as highlighted earlier in this report, can enable responsive and targeted responses. Such approaches have proven effective in addressing the HIV/AIDS epidemic in several countries.[22]

Separate mechanisms must also include accountability for nation states at a global level. We propose that heads of state should be accountable for their country's progress at the United Nations General Assembly on a biannual basis. This approach has been an important political component of the global fight to end HIV/AIDS, insofar as it has maintained global recognition and financial investment to

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address this disease. While the details of any national report card, would need to be drawn up to ensure stakeholder consensus, commitments on accountability should include progress towards key End TB milestones, other relevant sustainable development goals, as well as TB financing, adoption and implementation of WHO recommended policies, and registration of and access to the newest and best medical tools. [34] Table 4.9.1, gives an example of a report card, highlighting the current performance of ten high TB burden countries on several epidemiologic, programmatic, financial and multisectoral indicators.

Finally, OECD donor countries, international multilateral funding agencies, non-governmental funders, and the agencies of the United Nations, including WHO, UNICEF, and UNAIDS, all play vital roles in global efforts to end TB, for which they should also be held to account. Leveraging the Quality of ODA metrics already published by the Center for Global Development, Table 4.9.2 provides a report card that highlights strengths and weaknesses of major bilateral TB donors. This Commission proposes similar report cards for multilateral funders and major non-state actors so as to ensure that these institutions are also held account for their efforts, or lack thereof, towards ending the epidemic. Enhanced accountability of these institutions, not just to their board members or citizenry, but to TB survivors and their advocates in recipient countries, represents a global public good. While the indicators and governance for these proposed report cards will need to be agreed to by consensus, ~~dimensions~~ they should include performance monitoring and assessment, efficiency and effectiveness, sustainability, transparency, and responsiveness to corrective feedback.

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Notwithstanding ~~T~~the complexity of accountability, both semantically and practically, ~~notwithstanding,~~ ~~huge~~extraordinary opportunities exist to leverage digital data on many aspects of TB programming, impact, and finance to enhance accountability. Nonetheless, further ~~critical~~ assessments are~~is~~ necessary to determine how best to 'measure'[35] the accountability of TB stakeholders to ensure that it translates to actual progress towards ending the epidemic, and averting the millions more needless deaths.

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5.0 Conclusions

We can build a TB-free world. Many countries—~~.~~—even many low- and middle-income countries—~~.~~—have demonstrated that that it is achievable, ~~even in~~ despite ~~of~~ the limitations of existing tools. The prospect of a TB-free world is not a distant aspiration. It is a realistic objective that can be achieved with the right commitment of leadership and resources. It will be a difficult~~hard~~ task, with potential setbacks, including the challenge of drug-resistance, funding obstacles, and uncertainties about the correct~~right~~ prioritization of tools and implementation approaches. However, with targeted, evidence-based strategies, smart investments based on sound science, and a shared responsibility, we can end TB within a generation.

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Appendix

Figure 4.1. Conceptual Framework for Ending TB

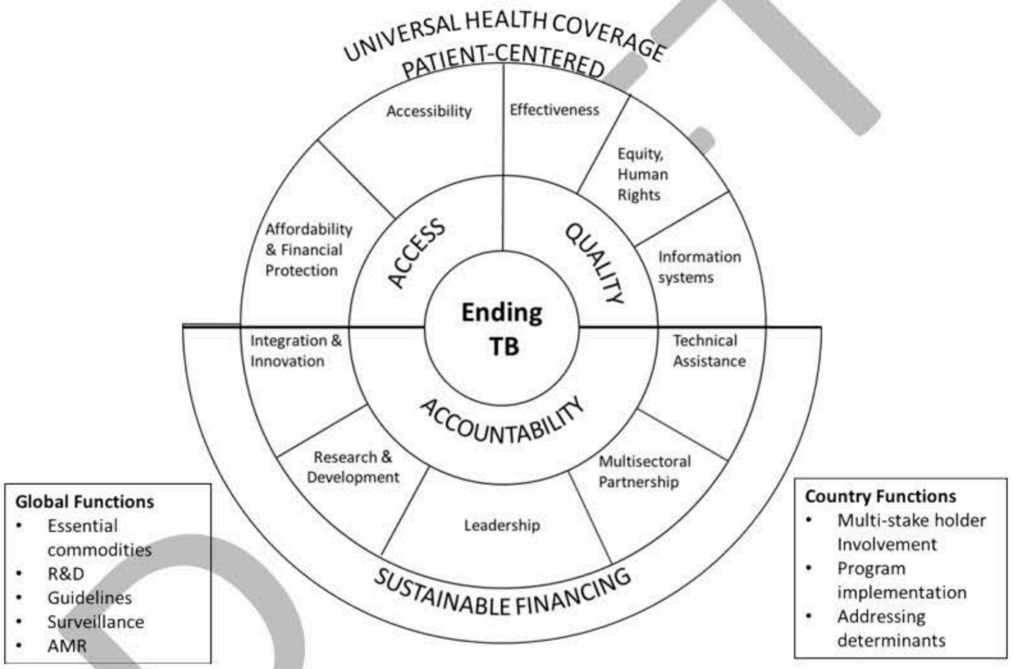

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Table 4.9.1 Country Report Card – 10 highest burden countries

TB Country Report Card																		
	Progress towards End TB targets		Monitoring & Management					Implementation		Financial Control				Tracers on equity, UHC and progress on relevant SDG indicators				
Indicator:	TB mortality: Projected year when country will reach End TB target based on current trends in decline in mortality[a(1)]	TB incidence: Projected year when country will reach End TB target based on current trends in decline in incidence[a(1)]	TB treatment coverage (2017)	DS-TB Treatment Success (2017)	Percent catastrophic costs related to TB[b]	LTBI treatment coverage (% PLWH who began TB preventive treatment; and % children under 5 yrs started on TB preventive treatment (in 2016))[b]		Implementation of Policy for DST for all bacteriologically confirmed patients(2)	Implementation of Policy for DST for all patients with RR-TB(2)	National public-sector financing: health as a proportion of total government expenditure[(3)]	World Bank ranking on public sector management	Proportion of TB expenditures from donor sources	Proportion health expenditure from out-of-pocket spending on health	Proportion of population covered by social protection system (SDG 1.3)[(4)]	Prevalence of under-nourishment (SDG 2.1)	Proportion of population with HIV that have access to ART (SDG 3.3)	Tobacco Taxation (% of retail price)[(5)]	Air Pollution (Concentrations of fine particulate matter [PM2.5])[(6)]
Benchmark:	Green: On target Yellow: Within 10 years of target Red: >10 years off target	Green: On target Yellow: Within 10 years of target Red: >10 years off target	Green: >90% Yellow: 80-90% Red: <80%	Green: >90% Yellow: 80-90% Red: <80%	0%	Green: >75% eligible started preventive therapy Yellow: 50-75% Red: <50%		Green: Yes Yellow: Yes, but not widely implemented Red: No or unknown	Green: Yes Yellow: Yes, but not widely implemented Red: No or unknown	Green: >15% Yellow: 10-15% Red: <10%	Green: >3.4 Yellow: 3.3-2.8 Red: <2.7	Green: <15% Yellow: 15-45% Red: >45%	Green: <15% Yellow: 15-45% Red: >45%	Green: >60% Yellow: 20-59% Red: <20%	Green: <5% Yellow: 5-20% Red: >20%	Green: >80% Yellow: 60-79% Red: <60%	Green: Yes Yellow: Yes, enforced and/or <25% tax Red: No tax or unknown	Green: <10 μg/m³ Yellow: 10-30 μg/m³ Red: >30 μg/m³
						PLWH	Pediatric (<5) HHC											
India	2120	2129	63%	72%		4.7%	1.9%			5%	3.7	26%	62.4%	11%	15%	49%	43.1%	62.4
Nigeria	2052	N/A	24%	84%		29%	18%			8.2%	2.8	74%	71.7%	4.4%	7%	30%	20.6%	38.8
Indonesia	N/A	2244	35%	85%		2.4%	1.5%			5.7%	3.2	39%	46.9%		8%	13%	57.4%	14.4
Bangladesh	2124	N/A	62%	93%			17%			5.7%	2.8	88%	67%	28.4%	15%	16%	77%	83.3
DR Congo	2190	2091	51%	89%			6.3%			11%	2.5	95%	38.8%	14.1%	30.5%	42%	40.9%	37.5
Pakistan	N/A	N/A	69%	93%						4.7%	3.1	90%	56.3%		20%	7%	60.3%	59.8
China	2183	2091	87%	94%						10.4%		3%	32%	63%	10%		50.8%	54.3
Philippines	2133	2091	58%	91%		49%	5%			10%		73%	53.7%	47.1%	14%	32%	62.6%	22.2
Tanzania	2163	2091	40%	90%		8.5%	31%			12.3%	3.4	94%	23.2%		32%	62%	33.9%	21.7
Myanmar	2060	2244	72%	87%		2.9%	1.9%			3.6%	3.0	84%	50.7%		17%	55%	35.3%	51.0

DS-TB=Drug Susceptible TB; LTBI=Latent TB Infection; HIV=Human Immunodeficiency Virus; PLWH=People living with HIV; HHC=Household Contact; RR-TB=Rifampicin Resistance TB; DST=Drug Susceptibility Test; ART=Anti-Retroviral Therapy

Note: Grey indicates unknown data.

[a]Projected years of reaching the End TB target were calculated based on the rate of decline in respective morality and incidence rates from 2000-2015, achieving 95% of the 2015 mortality rate and 90% of the 2015 incidence rate.

[b]Percent catastrophic cost data is currently unavailable. Much of LTBI treatment coverage is also unavailable, with only some data on HIV-infected persons and children living with TB-infected persons.

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Table 4.9.1 Bilateral Donor Report Card

	Commitment/Contribution to TB funding					Usage	Quality of Donor Aid**				Impact	
Donor	Total Dollars spent (2017)[1]	TB spending as a proportion of global DAH[1]	TB spending as a proportion of total country spending on DAH*[1]	TB spending as a proportion of total global DAH on TB*[1]	Trend (2011-2016)[1]	Output results assessed against targets?	Maximizing Efficiency[2]	Fostering Institutions[2]	Reducing burden[2]	Transparency[2]	Sustainability[1]	Relevance[3]
	$	%	%	%	Green: Increase >10% Yellow: No/little change (+/-10%) Red: >10% decrease	Yes, somewhat, not specified	QuODA ranking (out of 30)	QuODA ranking (out of 30)	QuODA ranking (out of 30)	QuODA ranking (out of 30)	% TB Funding spent on health systems support Green: >10% Yellow: 5-10% Red: <5%	Are the activities and outputs consistent with intended impacts?
UK	$246 million	0.66%	2.16%	1%	13%	Yes	10	6	10	18	11.6%	Yes, withholding 10% of funding to GF until 10 benchmarks for improvement are met[4]
USA	$475 million	1.27%	2.54%	8.8%	14%	Yes	24	20	31	15	12%	Yes
Japan	$69 million	0.18%	1.69%	0.2%	-2%	Not specified	16	4	25	22	2.5%	Not specified
Germany	$103 million	0.27%	0.93%	0.48%	25%	Not specified	30	11	22	16	16.7%	Not specified
France	$141 million	0.38%	0.47%	0.11%	23%	Not specified	14	8	26	25	2.8%	
Australia	$9.4 million	0.025%	0.54%	0.05%	-19%	Not specified	28	24	19	7	4.1%	
Canada	$85.7 million	0.27%	2.39%	0.33%	59%	No	11	12	21	1	12%	No

GF=Global Fund; IATI=International Aid Transparency Initiative; R&D=Research & Development; QuODA=Quality of Official Development Assistance; DAH=Developmental Assistance for Health

Green=Yes; Yellow=Somewhat; Red=No; Grey=Not specified

*Country spending on TB is only bilaterally focused and does not include funding to multilateral groups

**Quality of Donor Aid is based on the QuODA ranking of 30 donors, with each aid quality dimension being ranked individually (1 is highest ranking, 30 is lowest ranking).

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Panel 1: TB in children and adolescents

Recent modelling suggests that of the nearly 70 million children ~~are~~ estimated to be silently infected with *M. tuberculosis*, 1 million develop TB disease each year. Of the ~~one~~1 million, only one-third are diagnosed and treated; a quarter of a million die.[2,3] ~~It is estimated that~~Approximately 30,000 of the 1 million cases of TB in children are MDR TB,[4,5] but few such cases are diagnosed and appropriately treated. Infected children, especially adolescents, provide the seedbed for future cases of active TB. ~~Finally~~~~Tragically,~~ TB is among the top ten causes of death in children under age five–despite it being both preventable and treatable.[2,6]

TB in children was largely ignored until the last decade, when an increasing number of National Tuberculosis Programmes (NTPs) began developing paediatric guidelines and including children in TB control strategies. In 2015, the WHO End TB Strategy included children with an emphasis on prevention.[7] More recently, the first appropriately dosed, child-friendly, first-line formulations became available.[8]

What can be done now to address TB in children?

There is a critical need for new tools to address TB in children, particularly accurate, point-of-care diagnostics to detect both disease and infection; shorter, safer, simpler treatment regimens; and a better vaccine. However, in the meantime, ramping up the use of current tools now could dramatically reduce the burden of TB in children (Table P.1). Strategies and tools to prevent TB in children are underutilised, often ~~simply~~ due simply to a lack of availability. Moreover, infection control measures to protect children from infection in high-risk settings are implemented only rarely. Effective implementation of existing tools will require integrating other health services, especially those for maternal and child health.[9] TB screening and management can also be integrated into other services such as perinatal care, HIV clinics, or nutritional rehabilitation centres.[10-12]

While we must fully implement what we know works, increasing research on how to better prevent, diagnose, and treat TB in children must also be a priority. A significant increase in investment and sustained advocacy will be needed, but the return will be significantly more children alive and living TB-free.

Summary

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Childhood TB has received increased attention in the last decade, but TB still causes significant mortality and morbidity among this age group. While new diagnostics, treatments, and vaccines would help manage the paediatric TB epidemic, what we can and should do now is to integrate childhood TB management into other health services, implement existing interventions to their fullest, and increase our investment in research and development to save more young lives in years to come.

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Table P1: Interventions available now that could reduce the burden of childhood tuberculosis and priority areas for future research

Function of DAH	Interventions that could be done now	Future priority areas of research
Integration	• Better Integration - Maternal and child health services, malnutrition services, HIV services, antenatal and postnatal care • Incorporation of tuberculosis diagnosis and treatment into existing child health algorithms (IMCI)	• Cost effective models of integration and diagnostic referral pathways • Testing and validating optimal diagnostic algorithms at different age groups
Co-Morbidities	• HIV: Universal PMTCT, Universal ART for all children living with HIV, Preventive therapy (co-trimoxazole, isoniazid) • All children with malnutrition identified and treated	• Can community interventions for malnutrition reduce TB incidence in children?
Case-Finding	• Household contact investigations conducted following the diagnosis of all tuberculosis cases • Conducting outbreak investigations • Screening high-risk individuals: malnourished children, children living with HIV, children with other immunosuppressive conditions	• Role of new diagnostics including more specific skin test antigens in identifying children who need further investigation
Tuberculosis disease diagnosis	• Improved training of healthcare workers in making clinical diagnoses	• Development of point-of-care tests: To identify mycobaterial fragments in samples that are easier to obtain from children and/or to identify host-immune signatures that can differentiate tuberculosis from other diseases, and differentiate tuberculosis disease from infection

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	• Wider availability of good quality chest radiographs • Sharing of chest radiograph images electronically	
	• Wider availability of Xpert MTB/RIF Ultra	
Treatment of disease	• Use of FDC to treat drug-susceptible tuberculosis in young children • Implementation of the shortened regimen to treat multidrug-resistant tuberculosis in children	• Evaluate approaches to stratify children into those with more and less severe disease and treat them with different treatment regimens • Evaluation of shorter treatments for drug-susceptible disease • Evaluate shorter, injectable-sparing regimens for children with multidrug-resistant disease, including the use of novel agents • Evaluation of adjunct therapies
Bio-markers	• Wider availability of tests of infection (IGRA and TST) to be used in children to target preventive therapy	• Identify signatures that can identify children at increased risk of progressing from infection to disease • Identify biomarkers for diagnosis of disease
Treatment of infection	• Implementation of preventive therapy regimens: 3 months of daily isoniazid and rifampicin, 3 months of weekly isoniazid and rifapentine • Conducting outbreak investigations	• Evaluation of shorter regimens to treat drug-susceptible infection including with the novel agents • Evaluation of preventive therapy for contacts of multidrug-resistant tuberculosis
Vaccination	• Implementation of universal neonatal BCG vaccination with processes put in place to avoid disruptions to vaccine supply	• Development of novel vaccines (safer, more effective) to replace or augment the current BCG vaccine for newborns

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	• Development of novel vaccines to give to older children, children with disease or children following exposure

EPI: Extended program of immunizations, IMCI Integrated management of childhood infections, PMTCT: Prevention of mother to child transmission, ART: Anti-retroviral therapy, IGRA: Interferon-gamma release assay, TST: Tuberculin skin test, FDC: Fixed dose combination

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Panel 2: TB and stigma

Stigma stymies efforts to end TB: Stigma is a major barrier to ending TB. It deters people with TB symptoms from seeking medical care altogether or leads them to visit multiple or distant care providers or clinics, delaying a TB diagnosis.[13-17] Stigma also is associated with treatment non-adherence, poor patient-provider communication, and attrition from the TB care cascade.[15,16,18-20] Because of stigma, people with TB may conceal their illness from household members or fail to disclose details about their contacts, weakening the success of contact investigations.[21,22]

Stigma also contributes to catastrophic costs faced by people with TB. Negative public attitudes engender less support for disability benefits for TB patients,[23,24] leading some to hide their illness from employers or prolong their return to work,[25] which only aggravates financial hardships.[19,26] All told, stigma significantly diminishes the quality of life, social status, and mental well-being of people with TB.[19,27]

TB stigma is cultivated and sustained in law, policy, practice, and discourse: Stigma against TB is shaped by laws and social policies. Negative attitudes toward TB thrive in countries, including those of higher income, with punitive criminal justice policies, poor social protections, and targeted screening processes that frame certain vulnerable populations (e.g., migrants) as a health threat.[28-30] Health care policies that hold eliminating the risks of disease transmission above individual rights and freedoms denigrate the wellbeing of people with TB.[31,32] This commission asserts that TB programs must do more to protect the rights to privacy, security, health, and autonomy of those with and at risk for TB. Important lessons may also be learned from successes in reducing stigma against other diseases, such as HIV/AIDS.

1. ***Reducing TB stigma in government:*** Burdensome legal and social practices that systematically infantilize, impoverish, and expose people with or at risk for TB must be removed to end TB stigma.[29,33] To be successful, governments must lead the way by working with health institutions, workplaces, and communities to develop strategies to promote respectful attitudes and care for everyone.
2. ***Reducing TB stigma in communities:*** Public awareness campaigns that dispel fears and promote positive messages about TB can help reduce stigmatizing attitudes.[34,35 36] Campaigns that highlight the unfairness of obstacles faced by people who are sick tend to evoke public support for greater

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investment in the welfare of stigmatized groups.[37] On a smaller scale, engaging patients' families in the TB treatment process may reduce stigma within households.[38]

3. ***Reducing TB stigma in health institutions:*** Training and capacity building can reduce stigmatizing attitudes among healthcare workers[35,39,40], but occupational health concerns may allow exclusionary practices to continue unchecked.[41,42] Addressing workplace safety and instituting codes of conduct that model respect and privacy for patients are crucial steps towards dispelling fears and discouraging patient avoidance.[40]
4. ***Reducing shame in people with TB:*** Psychosocial interventions that deliver patient counseling, together with support networks such as adherence clubs, buddy systems, and home visits, have all been shown to reduce the shame some patients feel about having TB and increase treatment completion rates.[43-46] Economic interventions such as conditional cash transfers and patient work programs also can build resiliency to stigma,[47-50] especially among patients whose self-identity and social capital are linked to their ability to sustain their families and themselves.[51]

Stigma significantly thwarts TB elimination efforts. To overcome this barrier, this Commission asserts that we must move away from "control-based" mechanisms of treatment adherence, such as clinic-based DOT and forced hospitalization, and toward policies that promote treatment literacy, provider-patient partnerships, and socioeconomic and psychosocial support for people with TB. It may also be useful to learn from and model successful campaigns from HIV/AIDS, where community engagement, advocacy, and political buy-in have aligned to ensure that policymaking and program planning all, as a rule, mitigate stigma.

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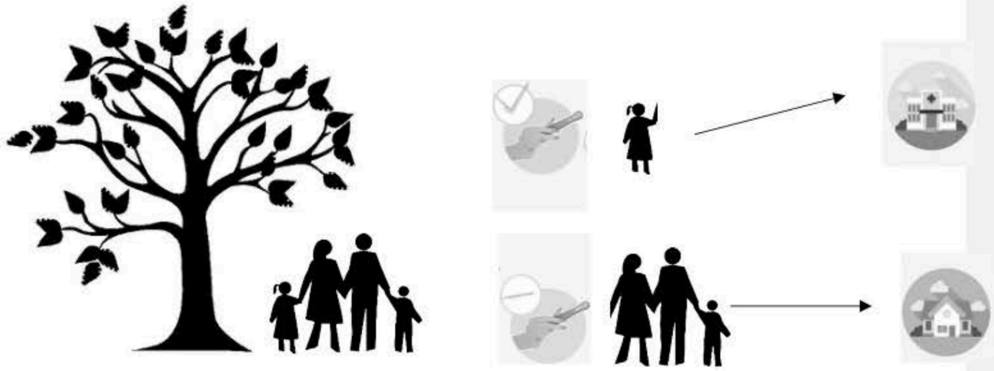
Panel 3. Potential impact of a TB Triage test



Panel 3. A triage test would enable active screening in communities or households, and passive screening where patients first seek care. Those with positive results would be referred for further work-up – a more efficient, patient-centered approach to case-finding than is currently available.

Most TB patients spend an excessive amount of time and money seeking answers--often they must miss work to journey to health facilities where it can take multiple visits or referrals before they learn they have TB. The toll this takes is evident: the roughly half of the total financial burden that TB patients bear – which averages about 30% of their annual income-- is accrued before diagnosis and treatment happens.[1] Millions are not diagnosed at all.

This is the result of a technical limitation – available TB tests are too complex and expensive to be used for basic screening. An easy-to-use, accurate rapid test is essential to reduce the costs and impacts patients face before they get to treatment.[11] A market analysis estimated that a total of 28.4 million triage tests are needed until 2020 to find undiagnosed~~missed~~ TB cases.

For the first time there are now five biomarker signatures in the R&D pipeline that are being validated for use in TB triage test development. With sustained funding for product development and clinical trials, a game-changing tool that would make a significant contribution to eliminating TB could be in hand within 3 years.

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Panel 4. The Life Prize

The Life Prize (formally the 3P Project) is a response to the unique challenges in TB research and development (R&D) and aims to create an enabling framework that promotes the development of affordable~~,~~ and accessible treatment regimens, working toward a pan-TB regimen of one month or less. The Life Prize approach (Figure) will transform the pathway for new TB drug and regimen development.

The key elements of the Life Prize Project are the innovative, collaborative open research framework with upfront as well as outcome-based funding and rewards, building stewardship, accessibility, and affordability goals into the development of a pan-TB treatment. The Life Prize will incentivize and reward scientists to bring promising new TB drugs into and through the drug development pipeline, and will facilitate the development of treatment combinations ensuring a ~~quicker and~~ more efficient way to develop TB treatments than the current system. To achieve this, the Life Prize consists of three elements:

- Prize funding for compounds entering clinical trials that fulfil predefined criteria.
- Additional grant funding to finance the development of the compounds in the Life Prize framework as part of new TB regimens in line with target regimen~~t~~ profiles, working towards a one month or less pan-TB regimen.
- All funding to require sharing of intellectual property and pre-clinical and clinical data to ensure open collaborative research and fair licensing for the competitive production of the final treatments.

The Life Prize compensates and rewards researchers and developers fully and upfront for their investments, thereby de-linking the financing of R&D from product prices and sales and promoting access and affordability as well as appropriate use of resulting products. The open sharing of data and intellectual property across Life Prize optimizes regimen development, ensuring data standardization in line with regulatory requirements to expedite the registration process and improve decision-making and resource allocation. The Life Prize aims to overcome some of the barriers that exist in TB drug R&D and enable existing actors to achieve more and

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encourage new actors into TB R&D. It also contributes to the AMR response by piloting a new model of R&D for a key AMR priority pathogen as well as stimulating early R&D into anti-infective agents.

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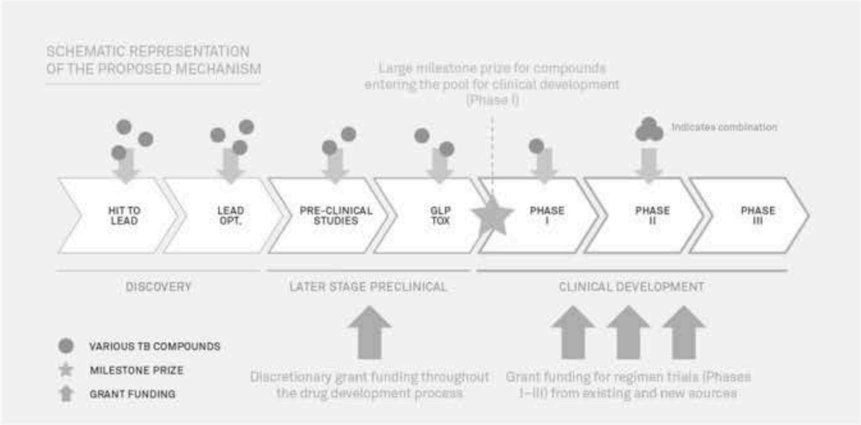
Panel 4: The Life Prize Schematic



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Panel 5 - South African investment case for TB

In order to support South Africa's National Strategic Plan for HIV, TB and STIs: 2017-2022, an investment case for HIV and TB was developed by the South African Department of Health and the National AIDS Council, with input from diverse stakeholders. The investment case for TB modelled the impact on TB incidence of scaling up the HIV 90-90-90 and TB 90-90-90 strategies alone or combination. The HIV and TB 90-90-90 strategies aim to find 90% of people living with TB and/or HIV, initiating treatment in 90% of those with TB and HIV, and attaining 90% treatment success for TB and viral suppression for HIV. The investment case suggested that scaling up the HIV and TB 90-90-90 interventions together using currently available tools will have the greatest impact on TB incidence (Figure 1) and reduce TB deaths by 87% over 20 years. ~~In order t~~To meet the WHO End TB targets by 2035, new tools will be required.

Implementing the TB 90-90-90 strategy will also require additional investment. At its peak in 2020-/21, the TB 90-90-90 strategy will require a doubling of the annual TB expenditure compared to the baseline scenario. Over 20 years, a 22% budget increase is required to implement the TB 90-90-90 strategy. Scaling up the combined TB and HIV 90-90-90 scenarios could potentially result in cost savings after 2035. The TB investment case provided the justification for the South African Treasury awarding South Africa's first-ever conditional grant for TB, which amounted to an increase in domestic funding for TB of ZAR500 million. The conditional grant has helped support the national roll-out of the short-course MDR TB regimen, with 4,083 MDR TB patients being treated with the short course regimen by February 2018.

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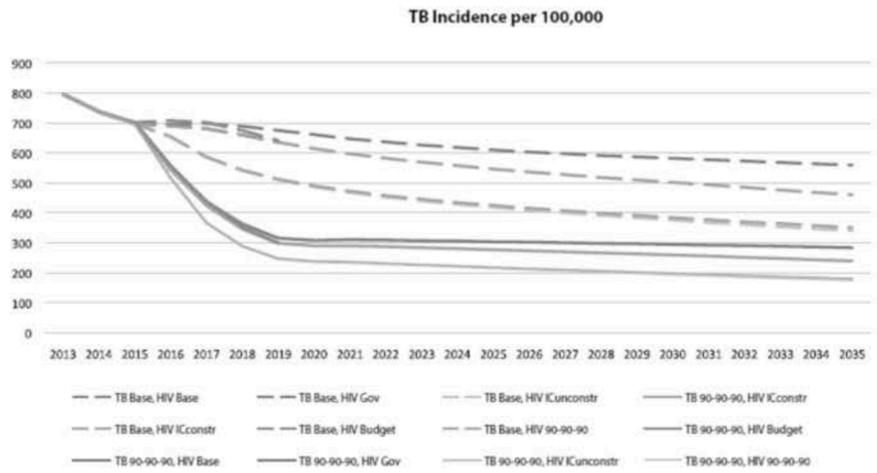

Figure 1A: Impact of scaling up HIV and TB scenarios
Footnote
HIV scenarios
1. Baseline (Scenario 1)
2. Government targets (Scenario 2): current mix of interventions against HIV and TB over the next 5 years, at *current* coverage targets endorsed by the government
3. Optimisation scenario without constraints (Scenario 3): The *most efficient mix* of interventions against HIV and TB. No budgetary limitations assumed
4. Optimisation scenario with budget constraints (Scenario 4): Outcomes assessed with the current budget envelope
5. Optimisation towards 90-90-90 targets (Scenario 5). The most cost-effective package of interventions to achieve the 90-90-90 targets.
6. 90-90-90 budget scenario (Scenario 6): The 90-90-90 scenario was adapted for a more budget-relevant scenario.
TB scenarios
1. Baseline scenario
2. 90-90-90 scenario, that aims to detect and treat 90% prevalent TB cases by 2020, 90% of those on treatment achieving treatment success.

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Panel 6: Long-term economic impact of TB on households in India

What is known and unknown: Multiple studies document the often substantial financial outlays faced early on as a result of catastrophic TB-related medical expenses.[2-5] However, few studies document the long-term economic impacts of TB on households or provide insights on how and how often TB causes impoverishment, and the potential for financial recovery.[6,7] Quantifying such impacts is important as the WHO targets 0% catastrophic expenditures for TB-affected families by 2030.[8]

What we did and found: We analyzed longitudinal data (26,032 rural households) from the India Human Development Survey (IHDS) I (2004-5) and II (2011-12). We used multivariable regressions to characterize the relationship between TB, expenditures, and loan-taking in the short-term and risks of impoverishment and debt in the long-term (7 years later), adjusting for baseline household sociodemographics and health as well as geographic and seasonal fixed effects. Findings are shown in **Table P.6**. Moderately poor households (<US$3.10/day per individual) reporting a case of TB at baseline were more likely to be extremely poor (<US$1.90/day per individual) 7 years later (TB: 36% [95% CI: 23% - 50%]; No TB: 21% [95% CI: 12% - 23%]), and extremely poor households were less likely to rise to moderate poverty (TB: 28% [95% CI: 22% - 34%]; No-TB: 36% [95% CI:35% - 37%], p<0·05). Despite India's overall economic growth during this period, 7-year growth of real, non-medical expenditures was three times higher for otherwise similar households without TB at baseline relative to those with TB. High-interest loan-taking was an important impoverishment mechanism. **Appendix YYY** documents the full methods and findings.

What it means: Households experiencing an active TB case at baseline are more likely years later to either remain poor or to become impoverished and indebted than comparable households without active TB. TB's adverse impacts, which extend well beyond an individual patient's health, are often long-lasting. These findings underscore the importance of WHO's social protection goals, which highlight the potential economic benefits of expanding social protection and insurance[4,9] and affordable credit to rural areas to prevent households affected by TB from remaining or becoming poor, -thus interrupting the disease-poverty cycle.[10]

AMERICAN OVERSIGHT

Table P.6 Relationship of poverty 7 years later to poverty at baseline

	Outcome (Predicted margin [95% CI])		p-value*
	No TB at baseline	TB at baseline	
Probability a household is in extreme poverty 7 years later			
Extreme poverty (baseline) (staying in extreme poverty)	0.39 [0.38 - 0.40]	0.41 [0.33 - 0.48]	0.57
Moderate poverty (baseline) (falling into extreme poverty)	0.21 [0.19 - 0.23]	0.36 [0.23 - 0.50]	0.02
Non-poor (baseline) (falling into extreme poverty)	0.12 [0.11 - 0.14]	0.15 [0.04 - 0.26]	0.65
Probability a household is in moderate poverty 7 years later			
Extreme poverty (baseline) (rising to moderate poverty)	0.36 [0.35 - 0.37]	0.28 [0.22 - 0.34]	0.01
Moderate poverty (baseline) (staying in moderate poverty)	0.34 [0.32 - 0.35]	0.32 [0.18 - 0.45]	0.78
Non-poor (baseline) (falling into moderate poverty)	0.22 [0.20 - 0.24]	0.33 [0.19 - 0.47]	0.14
Probability a household is non-poor 7 years later			
Extreme poverty (baseline) (rising to non-poor)	0.25 [0.24 - 0.26]	0.30 [0.21 - 0.38]	0.33
Moderate poverty (baseline) (rising to non-poor)	0.45 [0.43 - 0.46]	0.32 [0.18 - 0.46]	0.07
Non-poor (baseline) (staying non-poor)	0.64 [0.61 - 0.66]	0.52 [0.39 - 0.67]	0.13

7-year changes in household expenditures^^

	Rupees /year (Predicted margin [95% CI])		p-value*
	No TB at baseline	TB at baseline	
Total non-medical expenditures	15,344 [15,180 - 15,507]	4,211 [-4,501 - 12,923]	0.01
Total medical expenditures	2,933 [2,853 - 3,018]	-1773 [-6245 - 2,700]	0.04

Loan taking and reason for loan at baseline and 7 years later^^^ Outcome

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LANCET COMMISSION ON TUBERCULOSIS: PANELS

	(Predicted margin [95% CI])		p-value*
	No TB at baseline	TB at baseline	
Probability of having any loan at baseline	0.49 [0.49 - 0.50]	0.60 [0.53 - 0.66]	0.00
Probability largest loan is for medical expenses if having a loan at baseline	0.15 [0.15 - 0.16]	0.36 [0.26 - 0.46]	0.00
Probability of having any loan 7 years later	0.59 [0.59 - 0.59]	0.58 [0.51 - 0.66]	0.97
Probability largest loan is for medical expenses if having a loan 7 years later	0.18 [0.17 - 0.18]	0.25 [0.19 - 0.32]	0.00

^ The sample size is 26,032. A household is categorized as in extreme poverty, moderate poverty or non-poor if real per capita household consumption expenditure is <$1.90/day, ≥$1.90/day and <$3.10/day, or ≥$3.10/day respectively, after PPP adjusting real values in 2011 Rupees. Predicted margins were estimated following logit regression with controls for baseline poverty categories, TB at baseline, and their interaction and all other control variables. District and month fixed effects were included and household sample weights applied for each regression. Standard errors were clustered at the district level. We performed a Wald test of the equality of margins, post-estimation and report the p-value (Ho: No TB = TB).

^^ Outcomes are the predicted margins for changes in annual household expenditure (real 2011 rupees) between the baseline and 7 years later for specific spending categories and for No TB and TB households following multivariable ordinary least squares (OLS) regression and holding all else constant.

^^^ Outcomes are the predicted margins for loan-taking for No TB and TB households, following multivariable logistic regressions for baseline and follow-up outcomes, respectively and holding all else constant.

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LANCET COMMISSION ON TUBERCULOSIS: PANELS

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LANCET COMMISSION ON TUBERCULOSIS: PANELS

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Sat, 21 Apr 2018 20:47:31 +0000
To: Conrad, Patricia (NIH/NIAID) [E]
Subject: RE: Materials for 4/26 ICD Meeting 8:30-10:00

Thanks. Send a picture of "Conrad".

From: Conrad, Patricia (NIH/NIAID) [E]
Sent: Saturday, April 21, 2018 4:47 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: Re: Materials for 4/26 ICD Meeting 8:30-10:00

Icd is fine No more hhs Saudi meeting

Sent from my iPhone

On Apr 21, 2018, at 4:01 PM, Fauci, Anthony (NIH/NIAID) [E] (b)(6) wrote:

Is there no longer a conflict with overlapping times? Let us discuss.

From: Tabak, Lawrence (NIH/OD) [E]
Sent: Saturday, April 21, 2018 3:53 PM
To: (b)(6)
Subject: Materials for 4/26 ICD Meeting 8:30-10:00

Colleagues,

With apologies for the late arrival, please find the following items attached for our ICD Meeting on Thursday, April 26, from 8:30 – 10AM:

* Managing Bias in Official Duty Activities (background slides and ICD presentation)
* 2017-2018 Flu Season/Need for a Universal Flu Vaccine (background slides and ICD presentation)

* Agenda

These materials will also be posted on the ICD SharePoint site:
https://sps.od.nih.gov/ICdirector/default.aspx.

Please let me know if you have any questions.

Thanks, and best wishes,
Larry

<Lauer Bundesen Jaffe Steering Committee - Managing Bias in Official Duty Activities ICD 4 26 18 alternative short version.pptx>

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<Lauer Bundesen Jaffe Steering Committee - Managing Bias in Official Duty Activities ICD 4 26 18.pptx>
<IC Directors Meeting 4-26-18 Fauci.pptx>
<IC Directors Meeting 4-26-18 Background -Fauci.pptx>
<2018-04-26_IC Directors' Agenda[1].docx>

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From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Sat, 21 Apr 2018 20:01:08 +0000
To: Conrad, Patricia (NIH/NIAID) [E]
Subject: FW: Materials for 4/26 ICD Meeting 8:30-10:00
Attachments: Lauer Bundesen Jaffe Steering Committee - Managing Bias in Official Duty Activities ICD 4 26 18 alternative short version.pptx, Lauer Bundesen Jaffe Steering Committee - Managing Bias in Official Duty Activities ICD 4 26 18.pptx, IC Directors Meeting 4-26-18 Fauci.pptx, IC Directors Meeting 4-26-18 Background -Fauci.pptx, 2018-04-26_IC Directors' Agenda[1].docx

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Please let me know if you have any questions.

Thanks, and best wishes,
Larry

AMERICAN OVERSIGHT

Managing (Conflicts and) Bias in Extramural Staff Official Duty Activities

Michael Lauer MD, Liza Bundesen PhD, and Holli Beckerman Jaffe JDOffice of Extramural Research, National Institutes of Health (NIH) and NIH Ethics OfficeNIH IC Directors' MeetingApril 26, 2018Room 151, Building 1, NIH Main Campus, Bethesda, MD

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NIH National Institutes of Health
Office of Extramural Research

Conflict of Interest (COI) vs. Bias

COI

- Must be resolvedCompliance with criminal statutesSupervisor, DEC, and OD Ethics

Bias

- Must be managedIntegrity of extramural processSupervisor, DEA Director, and OER

- Official table last revised in 2008Practices are evolving and variable

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Case 1: Project Scientist on Cooperative Agreement

- Can a project scientist be a coauthor on All primary research papers?On a main results research paper?On any other research paper?Handle new applications from co-authors?

JAMA. 2002 Jul 17;288(3):321-33.

Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial.

Rossouw JE[1], Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J; Writing Group for the Women's Health Initiative Investigators.

Author information

JAMA FULL TEXT

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Case 2: Dual Roles on a Cooperative Agreement

- Program Officer, small IC with unique expertise in a niche area Recent R01 will be converted to a cooperative agreement Can s/he serve as Program Officer and Project Scientist?

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Case 3: Workshops => Publications => FOAs…

- NIH staff, extramural scientists plan state-of-the-science workshopCan NIH staff co-author a summary of the proceedings?

CellPress 10 YEARS 2005-2015

Cell Metabolism
Perspective

Understanding the Cellular and Molecular Mechanisms of Physical Activity-Induced Health Benefits

P. Darrell Neufer,[1,*] Marcas M. Bamman,[2] Deborah M. Muoio,[3] Claude Bouchard,[4] Dan M. Cooper,[5] Bret H. Goodpaster,[6] Frank W. Booth,[7] Wendy M. Kohrt,[8] Robert E. Gerszten,[9] Mark P. Mattson,[10] Russell T. Hepple,[11] William E. Kraus,[3] Michael B. Reid,[12] Sue C. Bodine,[13] John M. Jakicic,[14] Jerome L. Fleg,[15] John P. Williams,[16] Lyndon Joseph,[16] Mary Evans,[17] Padma Maruvada,[17] Mary Rodgers,[18] Mary Roary,[19] Amanda T. Boyce,[20] Jonelle K. Drugan,[20] James I. Koenig,[21] Richard H. Ingraham,[22] Danuta Krotoski,[23] Mary Garcia-Cazarin,[24] Joan A. McGowan,[20] and Maren R. Laughlin[17]

http://www.cell.com/cell-metabolism/fulltext/S1550-4131(15)00223-5

Funding Opportunity Title	Molecular Transducers of Physical Activity Preclinical Animal Study Sites (U01)
Activity Code	U01 Research Project – Cooperative Agreements

https://grants.nih.gov/grants/guide/rfa-files/RFA-RM-15-013.html

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Case 4: Writing Reviews or Commentaries

- SRO / PO involved in NIH activities to improve the rigor, reproducibility, and transparency of biomedical research S/he has been invited to write a commentary/review about the current state of the science in her IC's field with regard to rigor Acceptable?

The NHLBI Page

Predicting Productivity Returns on Investment

Thirty Years of Peer Review, Grant Funding, and Publication of Highly Cited Papers at the National Heart, Lung, and Blood Institute

Michael S. Lauer, Narasimhan S. Danthi, Jonathan Kaltman, Colin Wu

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http://circres.ahajournals.org/content/117/3/239

Case 5: Official Duties Involving Previous Collaborators

- PO led the development of an RFA, and several of the applicants happen to be her recent research collaborators from her previous job. S/he is referenced by name in several of the applications. Can s/he participate in the review meetings? Can s/he make funding recommendations?

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Case 6: Reviewing manuscripts

- Journal asks an NIH staff member to review a manuscript that was submitted by an NIH-funded scientist. Can s/he review the extramural scientist's manuscript?

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Case 7: Serving as an Expert Advisor

- Extramural investigators are drafting an NIMH grant proposal Asked OBSSR staff to serve as a named “expert advisor” He will not have influence over funding decisions. Can s/he serve in this “advisory capacity”?

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Case 8: Dual Roles with CRADAs

- A formal intramural scientist who was involved with a CRADA has moved to an extramural staff position. Can s/he be involved with applications, awards, or contracts that reference the CRADA or its intellectual property?

CRADA = Cooperative Research and Development Agreements

AMERICAN OVERSIGHT

Managing (Conflicts and) Bias in Extramural Staff Official Duty Activities

Michael Lauer MD, Liza Bundesen PhD, and Holli Beckerman Jaffe JDOffice of Extramural Research, National Institutes of Health (NIH) and NIH Ethics OfficeNIH IC Directors' MeetingApril 26, 2018Room 151, Building 1, NIH Main Campus, Bethesda, MD

AMERICAN OVERSIGHT

NIH National Institutes of Health
Office of Extramural Research

What are Official Duty Activities?

Extramural NIH interact, as part of their official duties, with other government and nongovernment scientists and with outside organizations.

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Elements of Official Duty Activities

- Performed by an employee using NIH time and resourcesMust be consistent with the authority and mission of NIHSometimes collaboration with outside individuals, organizationsAn intellectual endeavorNot other Outside Activities (e.g. clinical practice)

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Examples Include, but Not Limited to …

- Authoring manuscripts or other types of publicationsCollaborative research activities (e.g., cooperative agreements)Reviewing manuscripts for a journal or other publisherOrganizing a workshop or conferenceServing on a grant review panel for another government agency

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Conflict of Interest (COI) vs. Bias

COI

- Must be resolvedCompliance with criminal statutesSupervisor, DEC, and OD Ethics

Bias

- Must be managedIntegrity of extramural processSupervisor, DEA Director, and OER

- Official table last revised in 2008Practices are evolving and variable

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NIH Ethics Program

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- Ethics Policies
- Ethics Procedures
- Ethics Training
- DEC/EC Meetings
- Ethics Due Dates
- Resources
- *Prof Organizations

Official Duty Activities Involving Outside Organizations

Official Duty Activities are those activities performed by an employee as part of, or an extension of, regular official responsibilities. This discussion refers to official duty activities with an outside organization. The Standards of Ethical Conduct for Employees of the Executive Branch (at 5 CFR 2635) provide the basic guidelines for official duty activities, and the NIH sets the policy for implementing the guidelines at the NIH. An employee may participate in such activities only with advance approval as indicated below. For questions about specific activities, contact your IC's Deputy Ethics Counselor or Ethics Coordinator.

The activities an employee performs must be related to his/her official duties. Any official work performed with an outside organization must also be consistent with the authority and mission of the NIH. There should be compelling agency policy reasons for official duty activities with outside organizations. The activity must be avoided if the outside organization engages in lobbying or otherwise takes public positions on matters of significant controversy involving the NIH. Such activity should be limited where it is likely that the outside organization may become involved in legal disputes or other actions (e.g., medical care, personnel) that could subject it to liability. Because the activities are related to the employee's job, the employee may use non-confidential official information:

- that is available to the public or necessary to the work at hand, including information drawn from his/her current work or any work performed within the last 12-month period;
- associated with previous work (i.e., any matter in which the employee was involved prior to the last 12 months) regardless of whether it has been publicly disclosed; and
- that involves any on-going or announced NIH policy, program, or operation (rather than the outside organization's or the employee's general scientific or professional knowledge).

https://ethics.od.nih.gov/topics/official.htm

NIH Official Duty Policy Documents (all are in pdf format)

January 23, 2008, Memo from the NIH Deputy Ethics Counselor notifying ICs about the new policy. The policy consists of 3 primary elements:

- Introductory Statement: Official Duty Policy
- Conflicts Assessment Sheet
- Official Duty Activity table (1/23/08) of examples and approval level required.

See also the June 1, 2007, memo from the NIH Deputy Ethics Counselor to the IC Directors implementing the option to use a blanket official duty activity request to obtain permission for several official duty activities together. **Blanket Official Duty Activity Guidance and REVISED Blanket ODA Request Memo [Template]** is available on the forms page.

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2008 Chart that is being revised

OFFICIAL DUTY ACTIVITIES

BEFORE REFERRING TO THIS CHART: Consider if you have any personal outside interests or relationships with the outside organization involved in the proposed official duty. If you do, call your Deputy Ethics Counselor.*

This chart illustrates a variety of professional activities in which NIH extramural and intramural scientists often engage, but which, if considered official duties, may conflict with other official duties. This chart describes whether or not these activities are permitted as official duties, how these activities should be approved, and how any conflicts that might arise should be managed. As noted in the chart, most often these activities can be approved by the supervisor. Thus this chart should be used for guidance to steer the supervisor and employee towards the right decision regarding the approval of official duties and how to set up proper recusals when necessary. While the chart is quite comprehensive, there may be other activities not covered here that the supervisor and employee should manag accordingly.

KEY: E = Extramural Employee; I = Intramural Employee; B = Employees with both Extramural and Intramural Responsibilities

Activity	Not Permitted	No Formal Approval Needed	Supervisory Approval Needed	Other Clearance Needed	Notes
Writing and Editing Activities					
Review of manuscript (book, book chapters, or journal articles) where author is not grantee or collaborator		I	E, B		Consider the likelihood of author becoming a grantee or collaborator, and whether conflict would arise. Use supervisor's discretion on time between grant application and manuscript review but not less than one year.
Review of manuscript (book, book chapters, or journal articles) where author is or likely to be grantee or collaborator		I	E, B		**E, B:** if author is a grantee, then consider potential conflict if author is within employee's purview. Supervisor's discretion on time between grant application and manuscript review but not less than one year.
Submission of article to journal as author				E, B, I	Clear manuscript before submission to journal pursuant to IC's procedures.
Service on a journal's editorial board or editing a multi-author textbook (duties include publication decisions and scientific policy decisions)	E, I & B if position includes budget, management or personnel responsibilities		E, B, I		**E, B**: if author is a grantee, then consider potential conflict if author is within employee's purview. Supervisor's discretion on time between grant application and manuscript review but not less than one year.
Press releases and other media contacts				E, B, I	Consult with IC public affairs office after securing supervisory approval.
Materials to be submitted to Congress				E, B, I	Consult with IC legislative contact after securing supervisory approval.
Letters to the Editor/Op Ed articles			E, B, I		If writing in personal capacity (no use of NIH letterhead or title), no approval necessary. Caution not to disclose non-public information.

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Case Examples

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Case 1: Project Scientist on Cooperative Agreement

- Can a project scientist be a coauthor on All primary research papers?On a main results research paper?On any other research paper?Handle new applications from co-authors?

JAMA. 2002 Jul 17;288(3):321-33.

Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial.

Rossouw JE[1], Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J; Writing Group for the Women's Health Initiative Investigators.

Author information

JAMA FULL TEXT

Save items

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Case 1: Project Scientist on Cooperative Agreement

- No, should not automatically be a co-author on all papers For other papers, could be “probably not” or “maybe”:Level of involvement?Extramural scientists feel obligated? Equity between NIH staff?Creates appearance of (or real) bias?Applications: recusal for 3 years, avoid all review activities

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Case 2: Dual Roles on a Cooperative Agreement

- Program Officer, small IC with unique expertise in a niche area Recent R01 will be converted to a cooperative agreement Can s/he serve as Program Officer and Project Scientist?

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Case 2: Dual Roles on a Cooperative Agreement

- Very rarely: Should have different individuals Affects ability to make unbiased assessments of progressSupervisors should identify other individuals to oversee progress IC should develop a cooperative agreement management plan.

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Case 3: Workshops => Publications => FOAs…

- NIH staff, extramural scientists plan state-of-the-science workshopCan NIH staff co-author a summary of the proceedings?

CellPress 10 YEARS 2005-2015

Cell Metabolism
Perspective

Understanding the Cellular and Molecular Mechanisms of Physical Activity-Induced Health Benefits

P. Darrell Neufer,[1,*] Marcas M. Bamman,[2] Deborah M. Muoio,[3] Claude Bouchard,[4] Dan M. Cooper,[5] Bret H. Goodpaster,[6] Frank W. Booth,[7] Wendy M. Kohrt,[8] Robert E. Gerszten,[9] Mark P. Mattson,[10] Russell T. Hepple,[11] William E. Kraus,[3] Michael B. Reid,[12] Sue C. Bodine,[13] John M. Jakicic,[14] Jerome L. Fleg,[15] John P. Williams,[16] Lyndon Joseph,[16] Mary Evans,[17] Padma Maruvada,[17] Mary Rodgers,[18] Mary Roary,[19] Amanda T. Boyce,[20] Jonelle K. Drugan,[20] James I. Koenig,[21] Richard H. Ingraham,[22] Danuta Krotoski,[23] Mary Garcia-Cazarin,[24] Joan A. McGowan,[20] and Maren R. Laughlin[17]

http://www.cell.com/cell-metabolism/fulltext/S1550-4131(15)00223-5

Funding Opportunity Title	Molecular Transducers of Physical Activity Preclinical Animal Study Sites (U01)
Activity Code	U01 Research Project – Cooperative Agreements

https://grants.nih.gov/grants/guide/rfa-files/RFA-RM-15-013.html

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Case 3: Workshops => Publications => FOAs…

- No: signals that extramural scientists influence FOAsAlternatives: NIH staff, extramural scientists could author separate papers Limit influence of extramural scientistsRecusal period: Recused from applications with co-planners (not attendees) for three years from the date of the last substantial collaboration (e.g., day of workshop)

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Case 4: Writing Reviews or Commentaries

- SRO / PO involved in NIH activities to improve the rigor, reproducibility, and transparency of biomedical research S/he has been invited to write a commentary/review about the current state of the science in her IC's field with regard to rigor Acceptable?

The NHLBI Page

Predicting Productivity Returns on Investment

Thirty Years of Peer Review, Grant Funding, and Publication of Highly Cited Papers at the National Heart, Lung, and Blood Institute

Michael S. Lauer, Narasimhan S. Danthi, Jonathan Kaltman, Colin Wu

AMERICAN OVERSIGHT

http://circres.ahajournals.org/content/117/3/239

Case 4: Writing Reviews or Commentaries

- Maybe: Critical of ongoing research (including that not supported by NIH). Perceived as having bias for or against certain research groupsBalanced piece could support NIH's effortsFollow IC and NIH clearance procedures.

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Case 5: Official Duties Involving Previous Collaborators

- PO led the development of an RFA, and several of the applicants happen to be her recent research collaborators from her previous job. S/he is referenced by name in several of the applications. Can s/he participate in the review meetings? Can s/he make funding recommendations?

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Case 5: Official Duties Involving Previous Collaborators

- No. S/he should not attend the review meeting or make funding recommendations. After the awards are made, s/he cannot have former collaborator's grants in portfolio.Recusal period: three years from the end of collaboration, including publication of papers

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Case 6: Reviewing manuscripts

- Journal asks an NIH staff member to review a manuscript that was submitted by an NIH-funded scientist. Can s/he review the extramural scientist's manuscript?

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Case 6: Reviewing manuscripts

- Maybe:If the author is in the NIH staff member's portfolio, no. If not, yes, with supervisor's approval. Is this a necessary/important component of official duty; may be more appropriate as outside activity?Recusal period: One year

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Case 7: Serving as an Expert Advisor

- Extramural investigators are drafting an NIMH grant proposal Asked OBSSR staff to serve as a named “expert advisor” He will not have influence over funding decisions. Can s/he serve in this “advisory capacity”?

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Case 7: Serving as an Expert Advisor

- No:Even for staff who are not playing a role in review or funding recommendations, this could still give the appearance of the applicant trying to gain an advantage in review.

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Case 8: Dual Roles with CRADAs

- A formal intramural scientist who was involved with a CRADA has moved to an extramural staff position. Can s/he be involved with applications, awards, or contracts that reference the CRADA or its intellectual property?

CRADA = Cooperative Research and Development Agreements

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Case 8: Dual Roles with CRADAs

- No: could be perceived as influencing funding decisions that could ultimately provide her/him with personal financial benefitShould be recused from involvement with future applications from the research institutions involved in the CRADA.Recusal: Three years from the end of the collaboration.

CRADA = Cooperative Research and Development Agreements

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IC/O Clearance Procedures: Best Practices, Considerations

- Secure approval before engaging in activityIn the best interest of the Institute?An appropriate and necessary part of official duty?Are there concerns of COI (real or apparent) and/or bias? Collaborators in staff member's portfolio? Or likely to be? Would collaboration cause a reasonable person to question the impartiality of the staff member in his/her official duties?

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Management plan for cooperative agreements

- Disclosure / description of the bias How duality of interest will be managed, eliminated, or mitigatedStrategies to manage peer review activitiesBudgetary or programmatic lines of authority for an award PS/PO responsibilities in the Terms & Conditions of awardDocuments staff intent to publish (or not publish) with awardeeNeed for an ongoing review of the official's portfolio

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Other Discussion Items

- RecusalsHow long?When does the clock start?Governance for bias questions?Disclaimers?Other thoughts?

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A Few More Considerations

- Defining “substantial” scientific involvementDifferent “flavors” of project scientistsOver-use of cooperative agreement mechanismImbalance of staff workload – other PO’s pick up slackCherry-pick which projects become cooperative agreements

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Appendix Slides

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Why is this important?

- Preserve public trust in NIH's stewardship of public fundsProtect integrity of NIH and the research enterprise Have standard practices and policies at NIHWork collectively to identify potential conflicts or biasRecognize that there are a myriad of different situations that need to be addressed on a case-by-case basisSupport the best scienceComply with lawStatute, 18 USC § 208Regulations, 5 CFR parts 2635, 2640 and 5501

AMERICAN OVERSIGHT

Conflict of Interest and Bias in Official Duty Activities

Conflict of Interest (COI)When you have an outside (personal) interest, and you're assigned to participate in a government matter that affects that interest (two hats in the same matter)Can be a real conflict (financial) or an appearance (relationships) Defined in statute and regulationBiasPerspective, inclination, or opinion that can prevent impartial consideration of an issueDerived from personal/professional relationships

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What is a Real Conflict of Interest?

- Conflict arises when you wear two hats in the same matterYou always wear your government hat when engaging in an official duty activity, thus, must advance the government's interest You might also wear a personal, non-government hat, so you have a private interest, and that interest could be affected by the matter, for example:Stock in manufacturer of an investigational drugSpouse's bonus dependent on profitability of the company, and company's profit is tied to the grant you are administeringOfficer of scientific society that is co-sponsoring workshop

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What is an Apparent Conflict of Interest?

- When a person with who you have a “covered relationship” is a party to the government matter in which you want to participate.With whom do you have a covered relationship?Entities for which you served as an officer, director or employee within the last yearClose relatives (other than spouse and minor children)Entities for which your spouse currently serves as an officer, director or employee

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You Must Resolve the Real Conflict

Three ways:Divest (sell or resign) yourself of the outside interest. May not be possible, e.g., spousal employment or privately-held stockDisqualify (recuse) from the official duty matter. May need supervisor's concurrenceSeek a waiver through your ethics office. A very difficult and lengthy process.

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You Must Resolve the Appearance of a Conflict

- Three Ways:Disqualify (recuse) from the official duty matter. May need supervisor's concurrenceSeek an authorization through your ethics office. Likely, to allow at least some level of participation.Sever the relationship

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Conflicts Resolved, but Do You Have a Bias?

- **Writing or reviewing manuscriptsAnd author or co-author is a current granteeServing on a journal editorial boardAnd manuscripts are submitted by investigators in your portfolioTeaching as adjunct facultyAnd the institution where you teach submits applications to NIH**

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HHS-NIAID-20-0593-A-001884

Conflicts Resolved, but Do You Have a Bias?

- Serving on a grant review panelAnd PI on an application is your former mentorLeading an initiativeAnd several applicants to a FOA are your former collaboratorsParticipating in cooperative agreementsYou served as the Project Scientist, and the PIs will probably apply to future FOAs in your program area.

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What is Your Responsibility?

- Identify relationships with outside organizations Examples include:Organizations with whom you have a financial interestOrganizations with whom you have been recently employedIndividuals with whom you are currently collaborating, have recently published, or will have a future collaborationIndividuals you have recently mentored or have been mentored byIndividuals or organizations in your grant portfolio

AMERICAN OVERSIGHT

HHS-NIAID-20-0593-A-001886

What is Your Responsibility?

- Always consult with your supervisor and secure approval before proceeding with an official duty if you identify a COI or bias.As needed, consult your IC's DEC, DEA Director, and OER. Recognize that certain activities may create situations that require recusals or management plans.Work with your supervisor (and DEC or DEA Director, if applicable) to establish a recusal or management plan that explains the nature of the conflict or bias and the process for handling activities from which you are recused.

AMERICAN OVERSIGHT

HHS-NIAID-20-0593-A-001887

Ethics or Extramural Policy Management?

Managing official duties without risk for conflict or bias

Ensuring compliancewith criminal COI statutes;approving waivers and impartialityauthorizations

Protecting the integrity of theextramural business process; establishingrecusals and documentingmanagement plans to address bias.

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Guidance for Staff

- Be vigilant for the potential for conflict or bias.Talk to your supervisor early and often. Consult with your DEC, DEA director (and OD Ethics and OER as needed).Work with your supervisor to document decisions and management plans.Keep in mind . . . whether it's an issue of conflict or bias, final management decisions are up to your supervisor and IC/NIH leadership.

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How Do You Define Collaboration?

"Substantial involvement occurs when NIH scientific or program staff provides technical assistance, advice, coordination, and/or other functions above and beyond the usual level of programmatic and/or scientific stewardship for grants." NIH Manual Chapter 54815 – Implementation of Cooperative Agreements)

AMERICAN OVERSIGHT

In This Issue of JAMA

May 2, 2017
Volume 317, Number 17
Pages 1705-1812

Conflict of Interest

Edited by Howard Bauchner, MD, and Phil B. Fontanarosa, MD, MBA

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LETTERS

"Bias in the sense of a preformed judgement can grow out of many sources other than financial interest. Friendships, institutional associations, previous study and reflection, knowledge of a field, and life experience can all produce a predisposition to accept, reject, or interpret evidence in a certain way." -Harvey V. Fineberg, M.D., Ph.D., Gordon and Betty Moore Foundation

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IC Directors Meeting

Chasing Influenza: The Need for a Universal Influenza Vaccine

Anthony S. Fauci, M.D.
Director
National Institute of Allergy and Infectious Diseases
National Institutes of Health

April 26, 2018

AMERICAN OVERSIGHT

- Current seasonal influenza vaccines are not consistently effective
- Pandemics do occur and response after the fact is not effective
- "Chasing after" potential pandemic outbreaks (pre-pandemic viruses) is costly and ineffective

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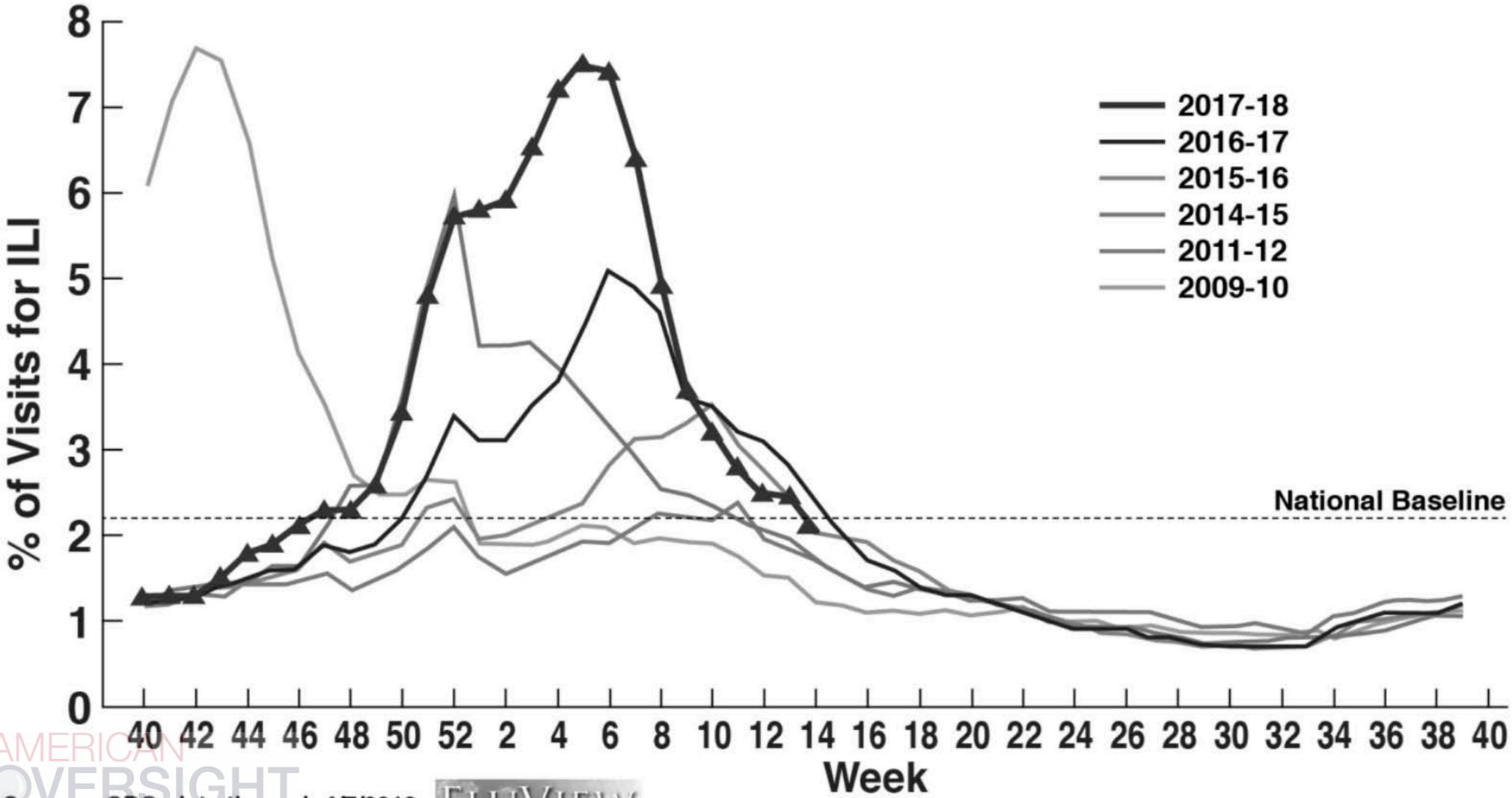
Weekly Reporting of Influenza-Like Illness in the United States by Season



Source: CDC, data through 4/7/2018 FLUVIEW

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Adjusted Influenza Vaccine Effectiveness Estimates, United States

*Interim estimate

Source: CDC

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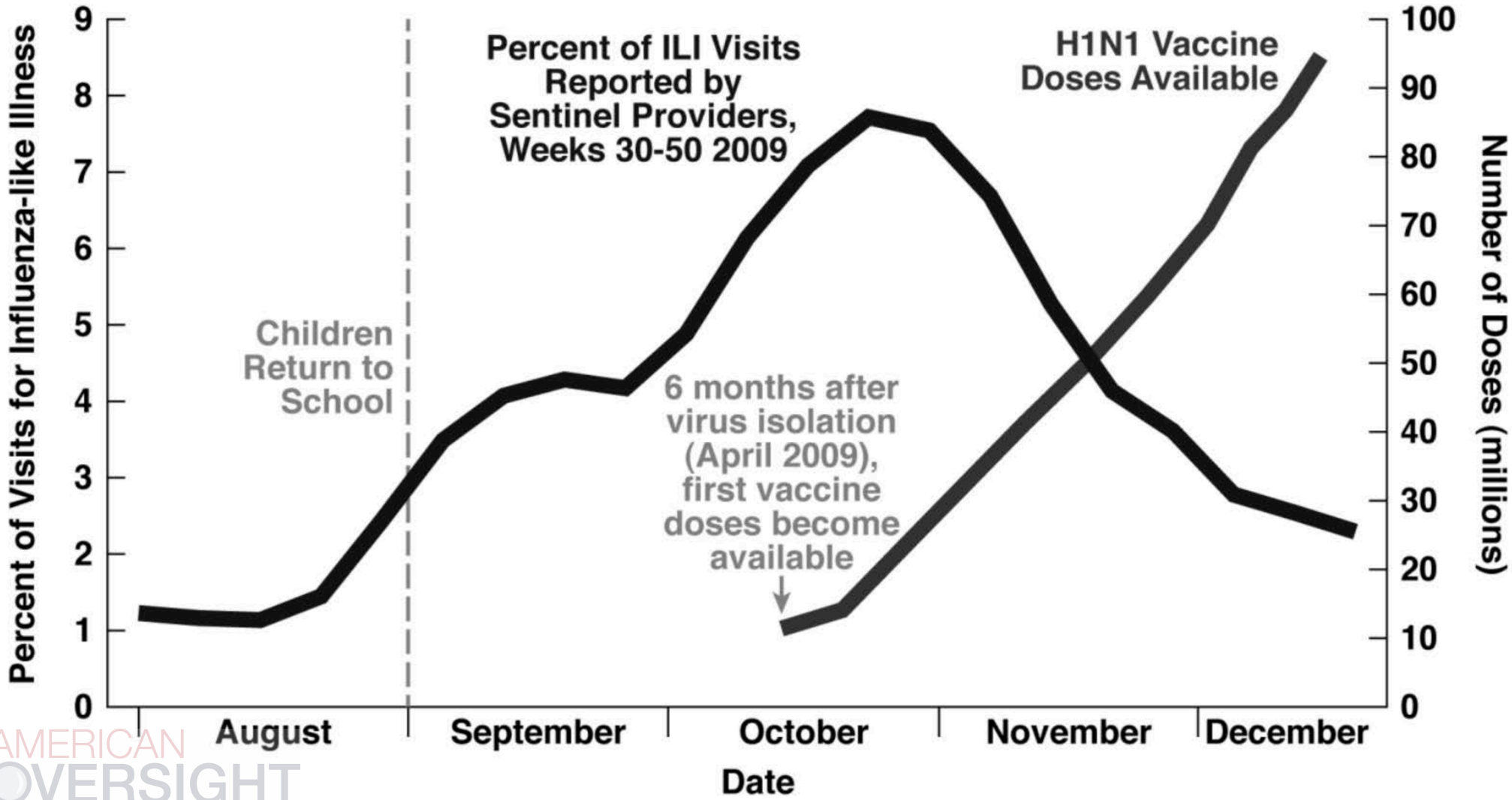
Vaccine Lags Behind 2009 H1N1 Influenza Pandemic



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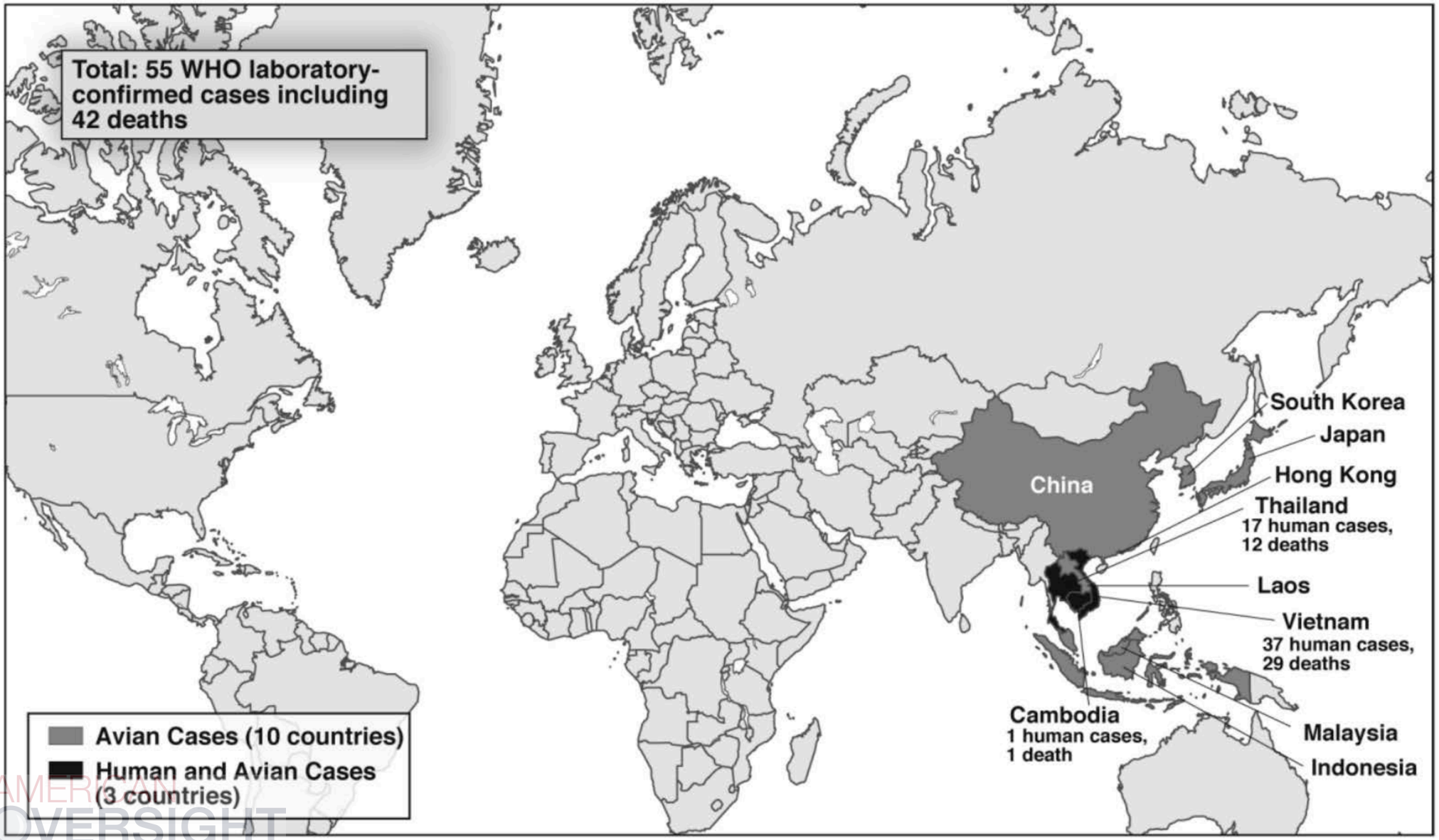
H5N1 Influenza Cases, 2003-2005



Source: WHO 2/2005

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HHS News

U.S. Department of Health and Human Services
FOR IMMEDIATE RELEASE
Tuesday, September 21, 2004

www.hhs.gov/news

HHS Orders Avian Flu Vaccine Doses as Preventive Measure

HHS Secretary Tommy G. Thompson announced today the awarding of a contract to Aventis Pasteur Inc. to manufacture and store 2 million doses of avian influenza H5N1 vaccine, an important initial acquisition to better prepare the nation for an influenza pandemic.

The New York Times

November 1, 2005

Bush Calls for $7.1 Billion to Prepare for Bird Flu Threat

President Bush today unveiled a strategy to combat the threat of an avian flu pandemic, calling for $7.1 billion in emergency spending to stockpile reserves of medicines and to press ahead with the development of a new vaccine.

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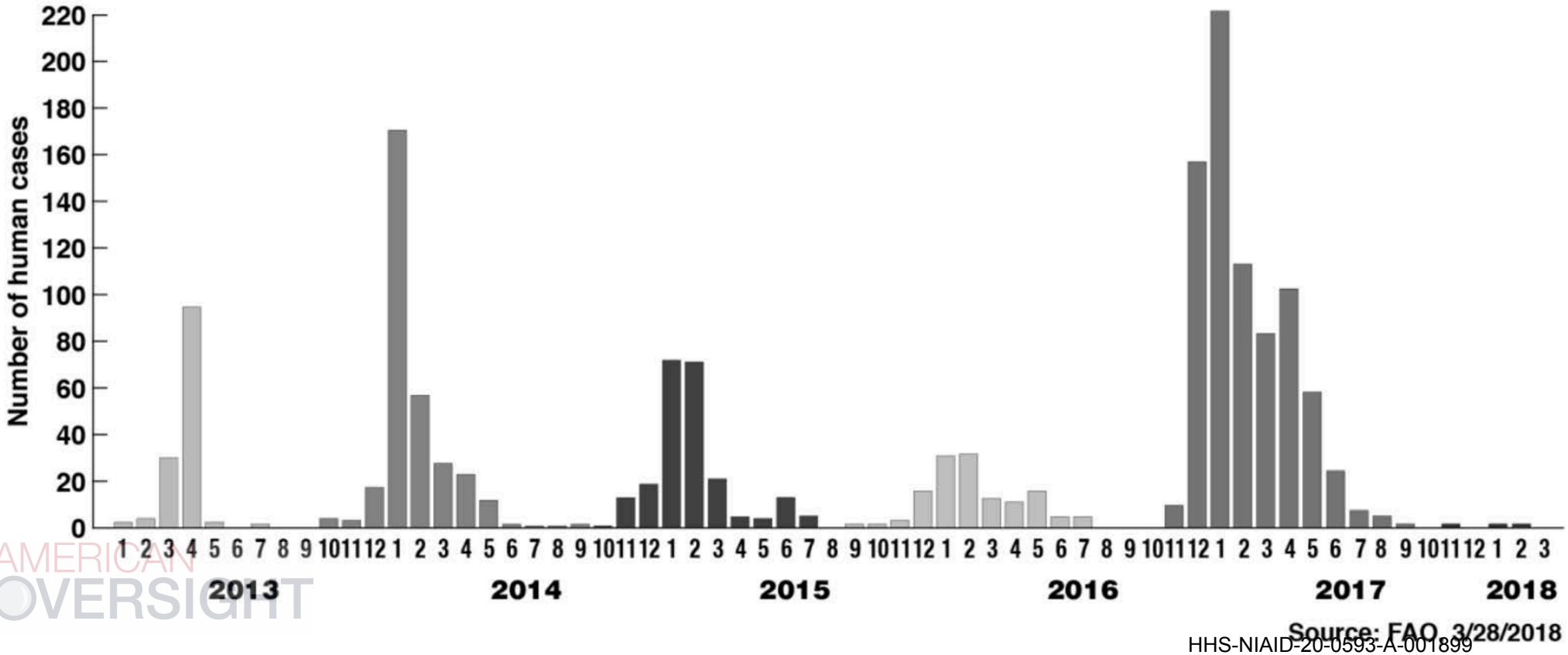
Waves of Human H7N9 Influenza Infections in China, February 2013-present

- **1,625** confirmed human cases, **622** deaths
- 5th wave: >50% of cumulative cases



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STAT

May 1, 2017

As Bird Flu Spreads, US Concludes Its Vaccine Doesn't Provide Adequate Protection

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National Institutes of Health
Turning Discovery Into Health

FOR IMMEDIATE RELEASE
Thursday, March 15, 2018

News Release

H7N9 Influenza Vaccine Clinical Trials Begin

- Inactivated 2017 H7N9 influenza vaccine manufactured by Sanofi Pasteur with or without AS03 adjuvant
- Phase 2 trials will enroll up to 420 volunteers ages 19 to 64 years and up to 300 volunteers >65 years of age

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Major Challenges in Influenza Vaccinology

- Production of vaccines – Egg-based versus Cell-based versus Recombinant DNA Technology

- Strain-Specificity versus Universal Strain Coverage

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Evolution of Technologies for Influenza Vaccines

Egg-based → Cell-based → Recombinant DNA Technologies

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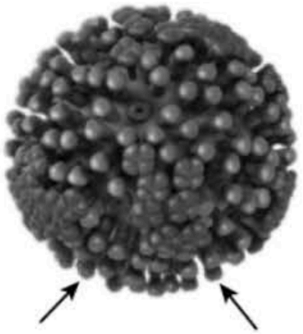
Vaccine Platform Technologies

Genetic immunization (DNA, RNA)

Viral protein immunization



Flu Virus Hemagglutinin (HA) [H1, H5, H7]

Genetic Immunization (DNA and RNA vaccines)

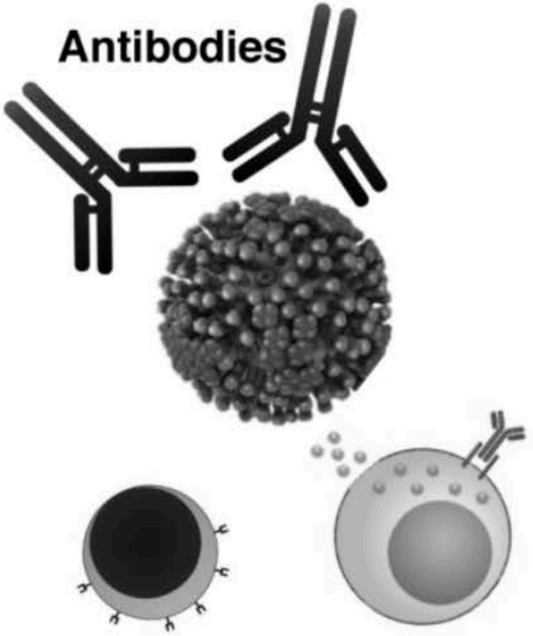
Viral vector (cold virus: adenovirus)

Recombinant protein

Nanoparticles (protein on particle)

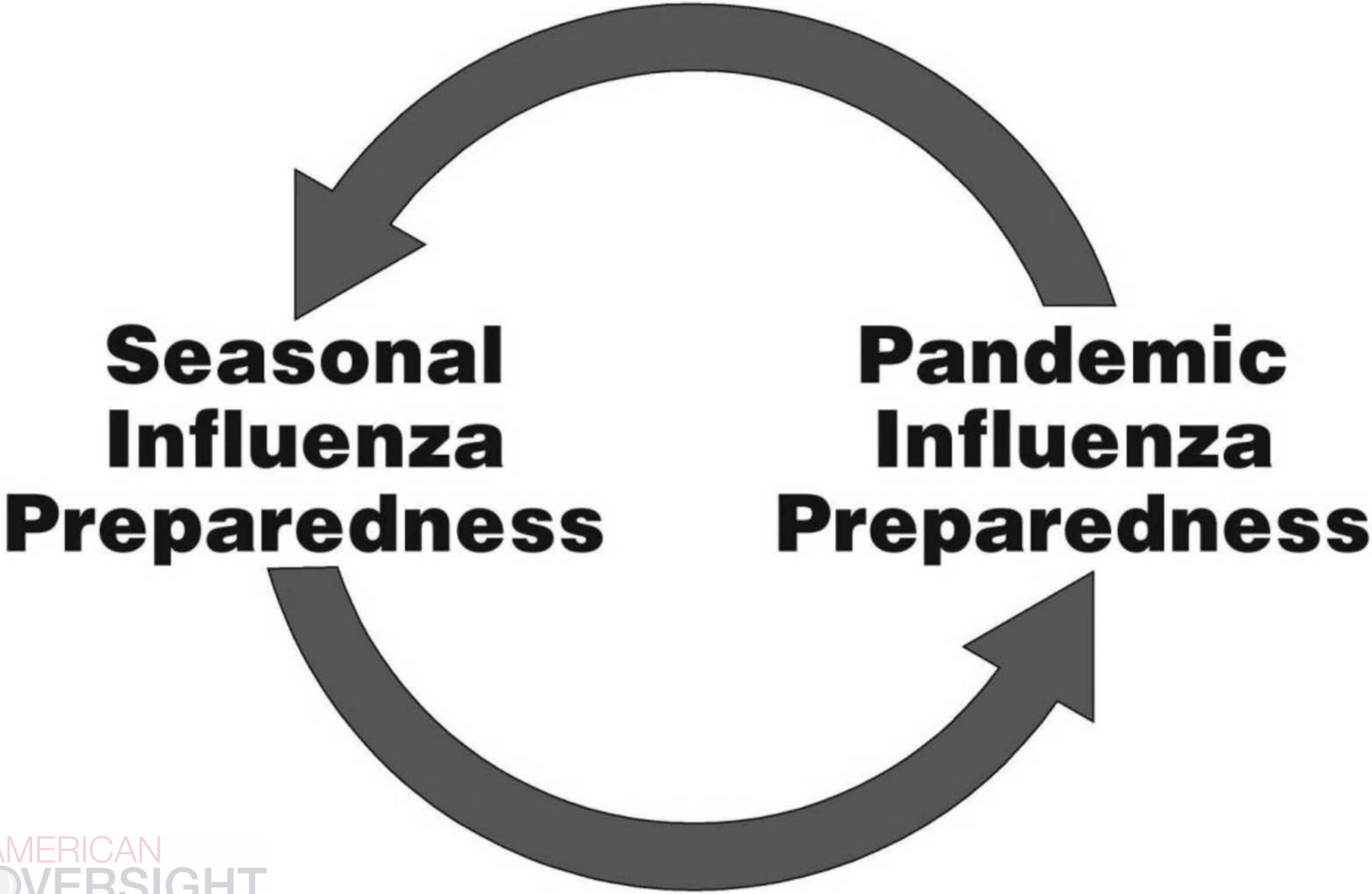
Virus-like particle (VLP) (no RNA; non-infectious)

Stimulate the body's immune system to attack the virus



Courtesy of VRC

AMERICAN OVERSIGHT



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The
New England
Journal of Medicine
Established in 1812 as THE NEW ENGLAND JOURNAL OF MEDICINE AND SURGERY
Volume 378 January 4, 2018 Number 1

Chasing Seasonal Influenza — The Need for a Universal Influenza Vaccine

CI Paules, SG Sullivan, K Subbarao, and AS Fauci

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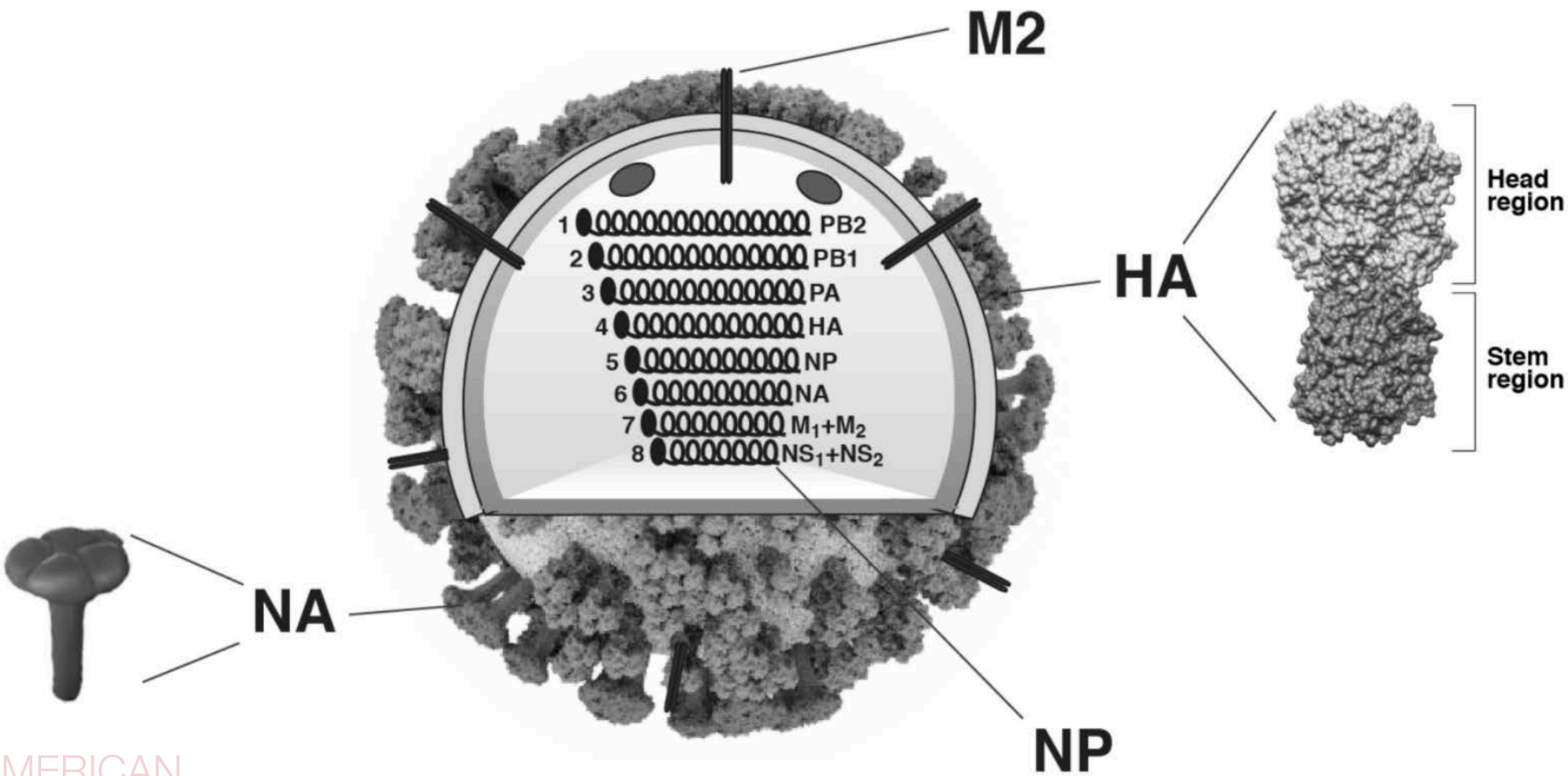
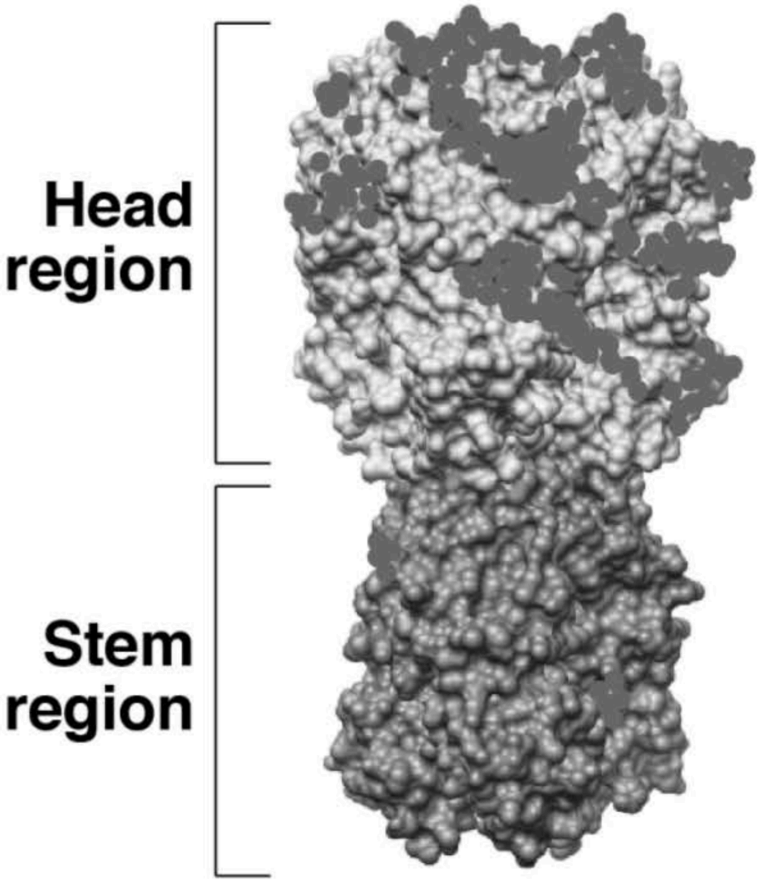
Selected Targets for "Universal" Influenza Vaccines



AMERICAN OVERSIGHT

Courtesy of VRC

Influenza A Hemagglutinin (HA)



Head region

- Target of current influenza vaccines
- Differs among influenza strains
- Many mutations (●) each season

Stem region

- Target of universal influenza vaccines
- Similar among influenza strains
- Few mutations each season

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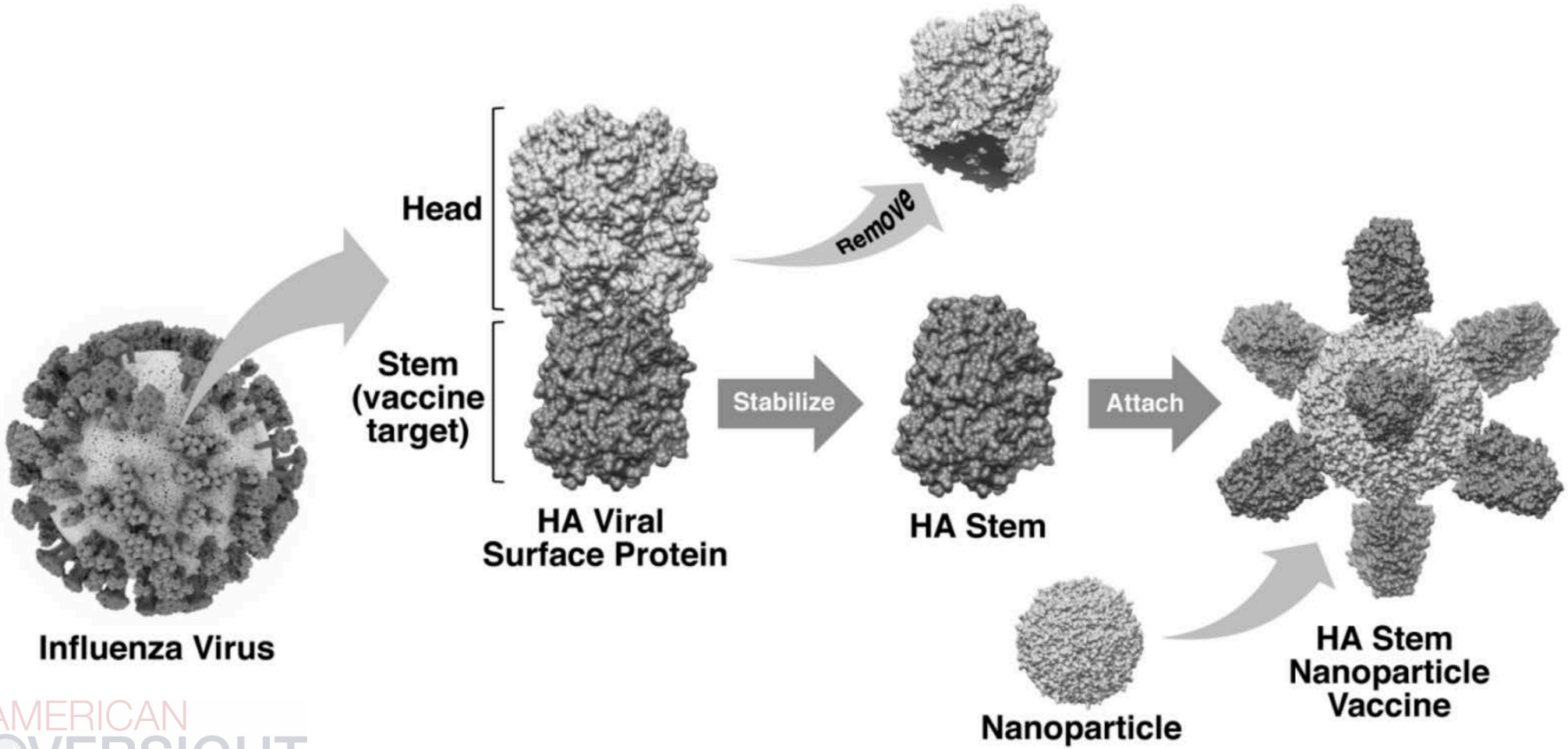
Courtesy of VRC

Representative Approach to the Development of a Universal Influenza Vaccine



Courtesy of VRC

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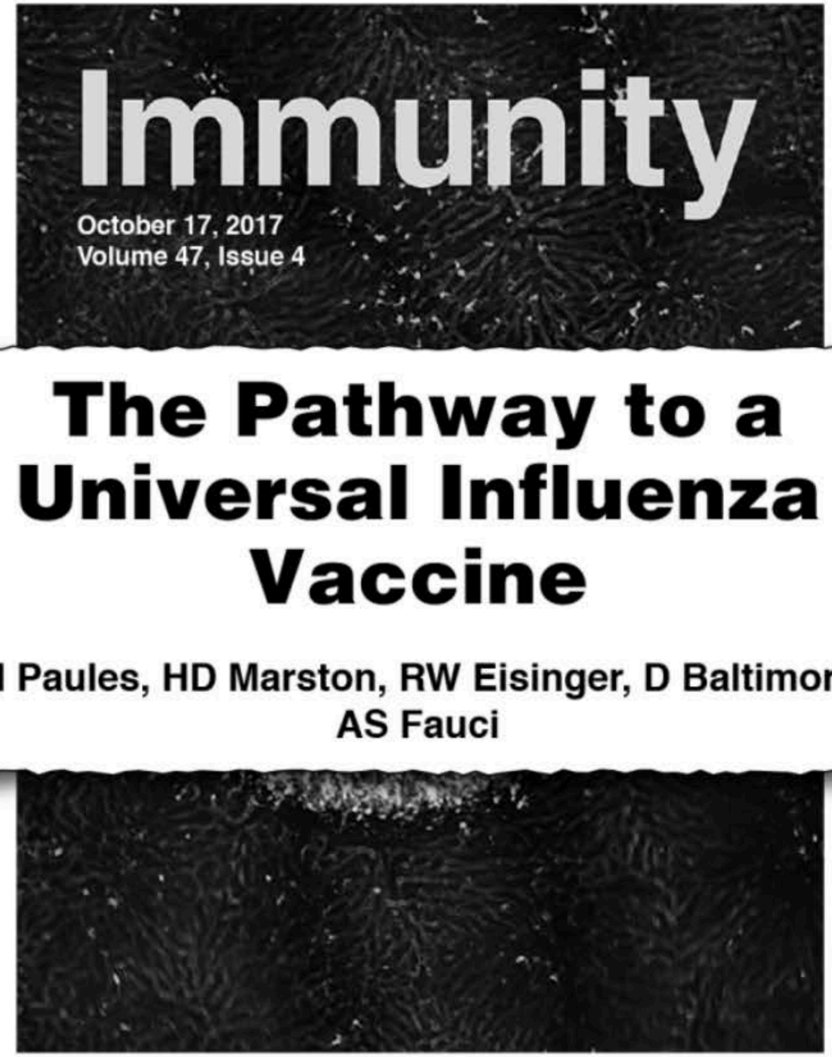

The Pathway to a Universal Influenza Vaccine

CI Paules, HD Marston, RW Eisinger, D Baltimore, AS Fauci

AMERICAN OVERSIGHT

Published online
February 28, 2018

The Journal of Infectious Diseases

IDSA Infectious Diseases Society of America

hivma hiv medicine association

A Universal Influenza Vaccine: The Strategic Plan for the National Institute of Allergy and Infectious Diseases

EJ Erbelding, D Post, E Stemmy, PC Roberts, A Deckhut Augustine, S Ferguson, CI Paules, BS Graham, AS Fauci

AMERICAN OVERSIGHT

- **Three key research areas:**
  - Improve understanding of transmission, natural history, and pathogenesis of influenza infection
  - Precise characterization of influenza immunity and correlates of immune protection
  - Support rational design of universal influenza vaccines

IC Directors Meeting

Chasing Influenza: The Need for a Universal Influenza Vaccine

Anthony S. Fauci, M.D.
Director
National Institute of Allergy and Infectious Diseases
National Institutes of Health

April 26, 2018

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Influenza A Viruses

- Single-stranded, segmented RNA viruses
- Present in a wide variety of birds and mammals
- Wild waterfowl are natural hosts
- Can cause serious disease in humans

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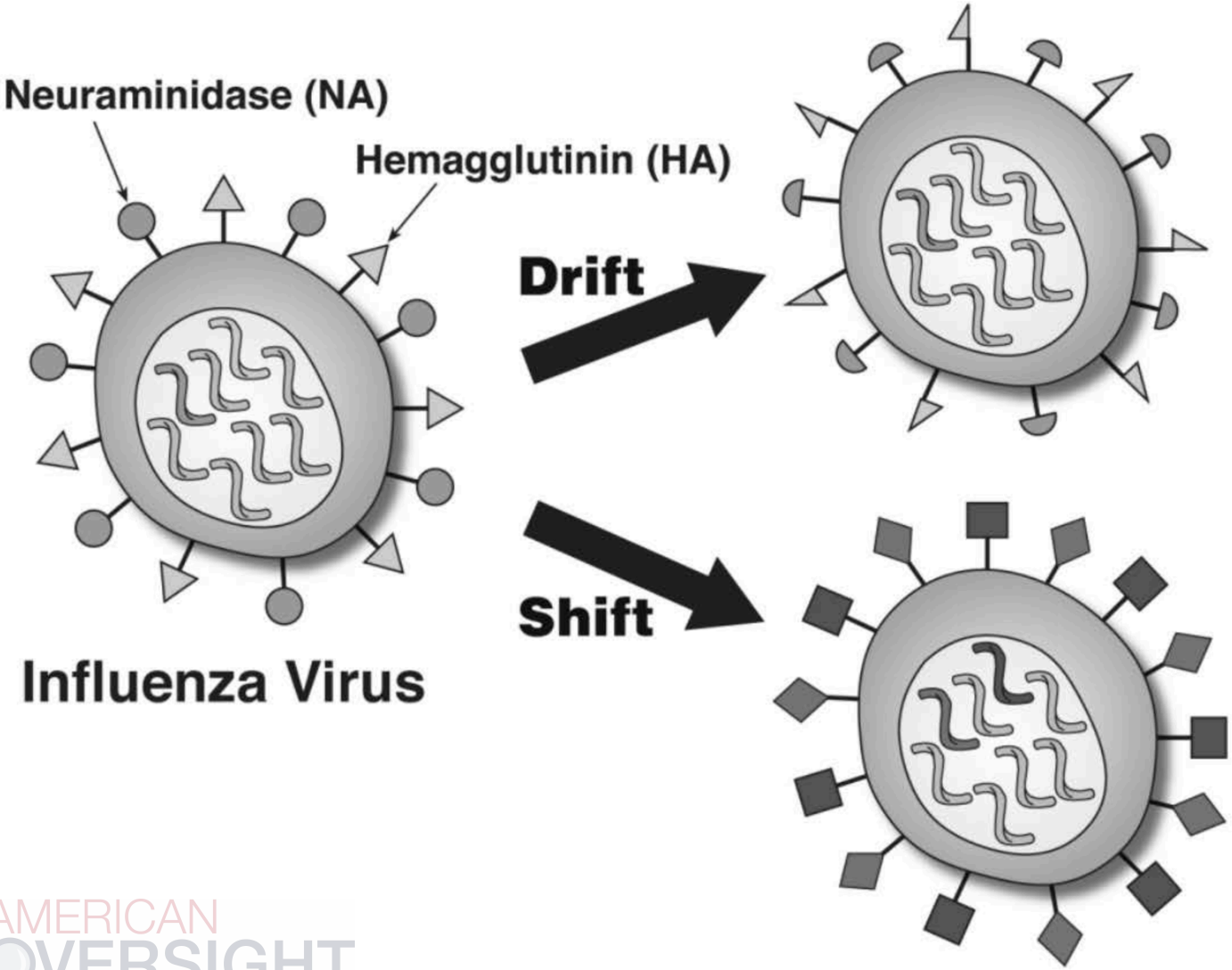
Seasonal vs. Pandemic Influenza



Seasonal Influenza

- Annual occurrence
- Residual immunity in population

Pandemic Influenza

- Unpredictable rare occurrence
- "Naïve" population

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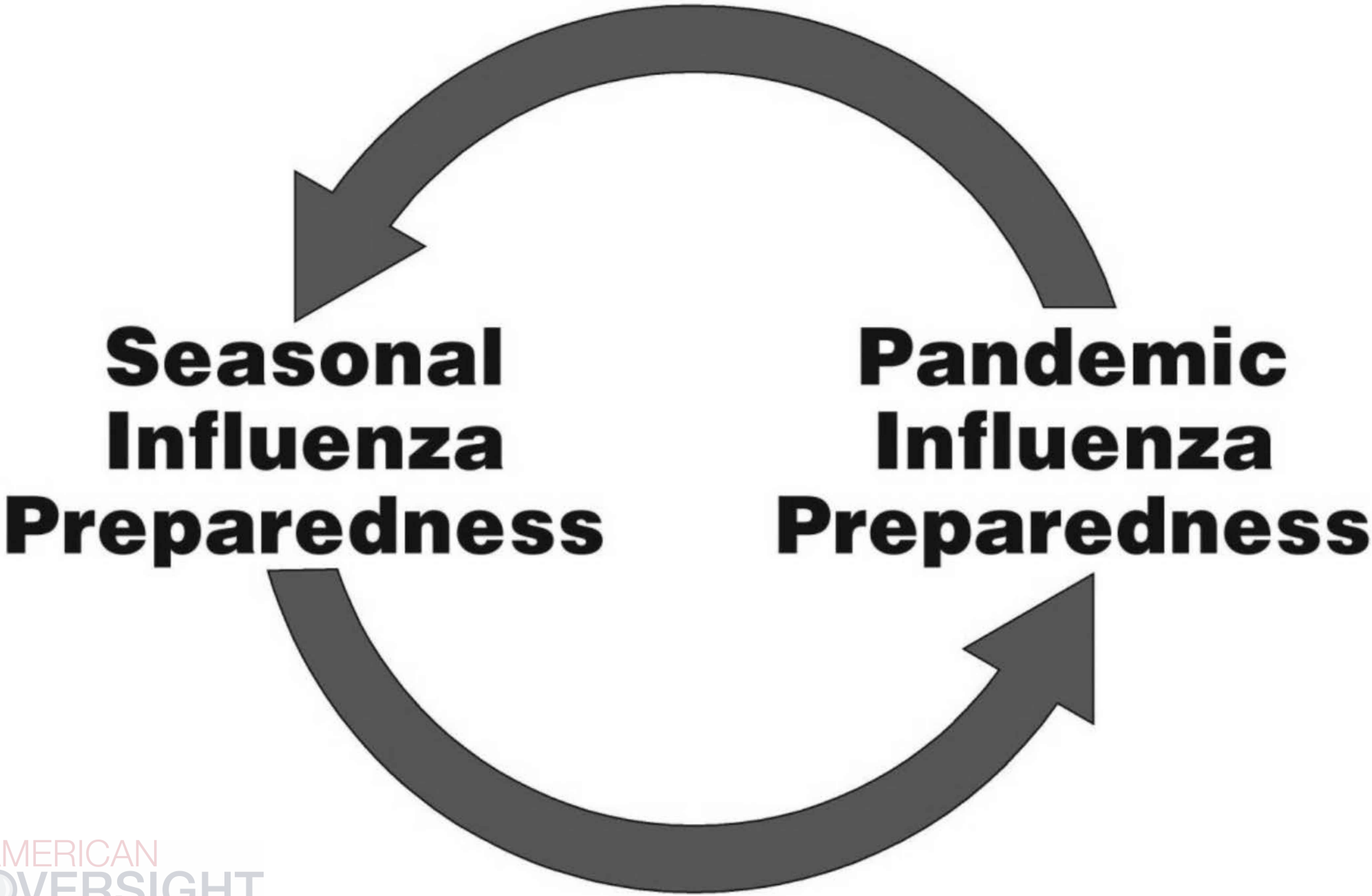

AMERICAN OVERSIGHT

- Current seasonal influenza vaccines are not consistently effective
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- "Chasing after" potential pandemic outbreaks (pre-pandemic viruses) is costly and ineffective

AMERICAN OVERSIGHT

- **Current seasonal influenza vaccines are not consistently effective**

- **Pandemics do occur and response after the fact is not effective**

- **"Chasing after" potential pandemic outbreaks (pre-pandemic viruses) is costly and ineffective**

AMERICAN OVERSIGHT

Adjusted Influenza Vaccine Effectiveness Estimates, United States

*Interim estimate

Source: CDC

AMERICAN OVERSIGHT

Antigenic mismatch is a well known cause of decreased seasonal influenza vaccine effectiveness...

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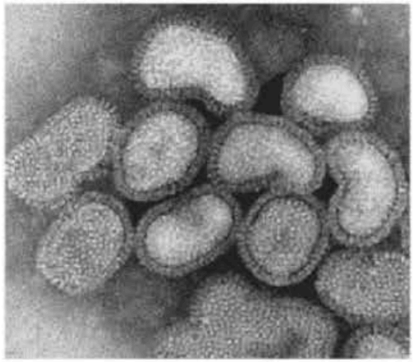
Seasonal Influenza Vaccine Development

February/March → April - July → August - September



Virus Selection
(WHO, FDA)

Production
Testing
Filling/Packaging

Product Release/ Shipping

Vaccination Begins

- Immunity develops approximately 2 weeks after vaccination

6-7 months

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Adjusted Influenza Vaccine Effectiveness Estimates, United States

*Interim estimate

Source: CDC

AMERICAN OVERSIGHT

THE BALTIMORE SUN

December 12, 2014

Flu Vaccine Still Advised Despite 'Drifting' of One Strain of Virus

Pittsburgh Post-Gazette

December 22, 2014

Vaccine Mismatch, Flu Afflict Region

INDEPENDENT

February 6, 2015

Flu Vaccine Given to Millions This Winter is Basically Useless

AMERICAN OVERSIGHT

but what about years where the match is a good one?

AMERICAN OVERSIGHT

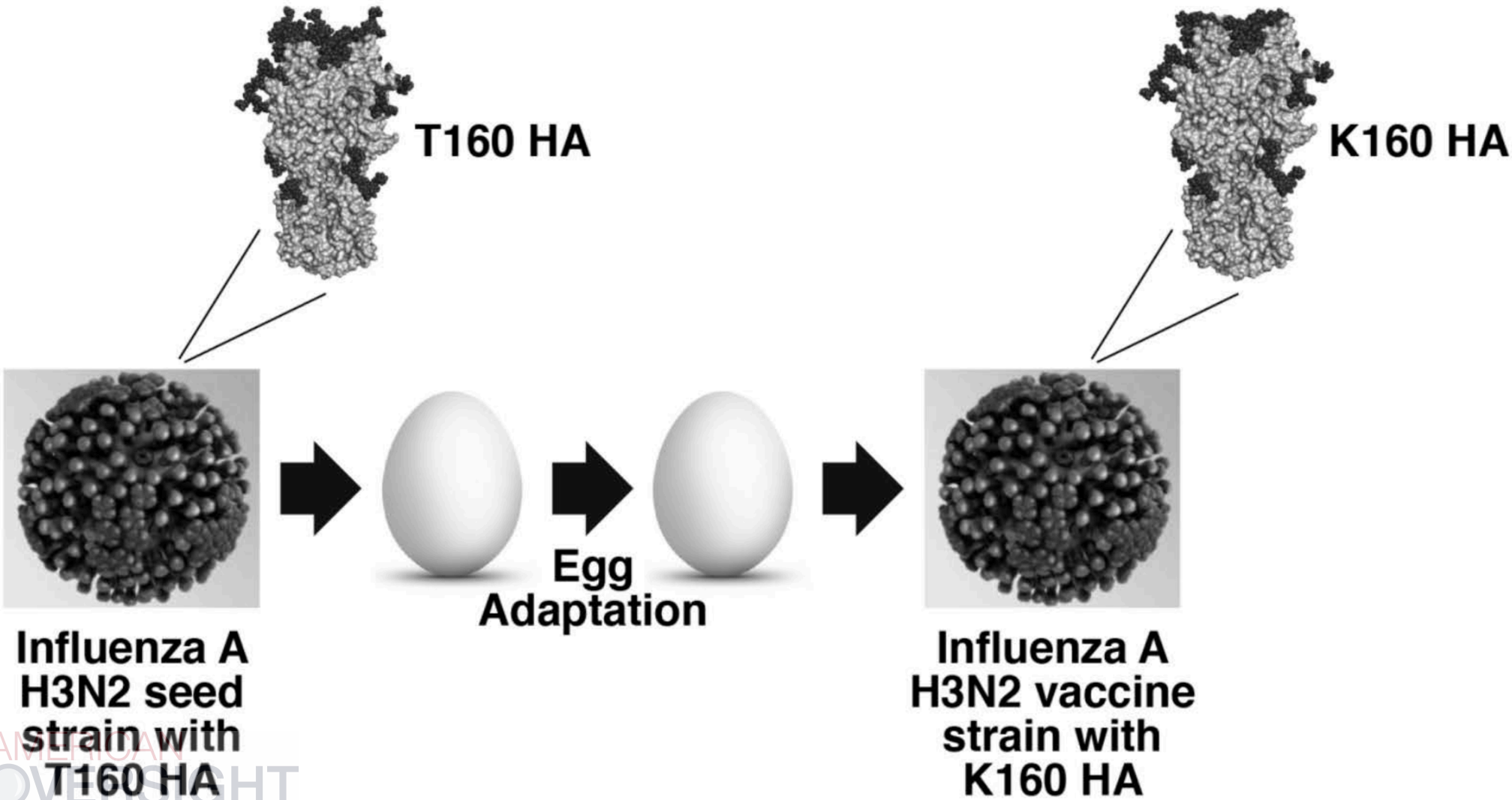
Adjusted Influenza Vaccine Effectiveness Estimates, United States

*Interim estimate

Source: CDC

AMERICAN OVERSIGHT

Egg Adaptation of Influenza A H3N2 May Contribute to Decreased Vaccine Effectiveness



AMERICAN OVERSIGHT

What happened during the 2017-2018 flu season in the United States?

AMERICAN OVERSIGHT

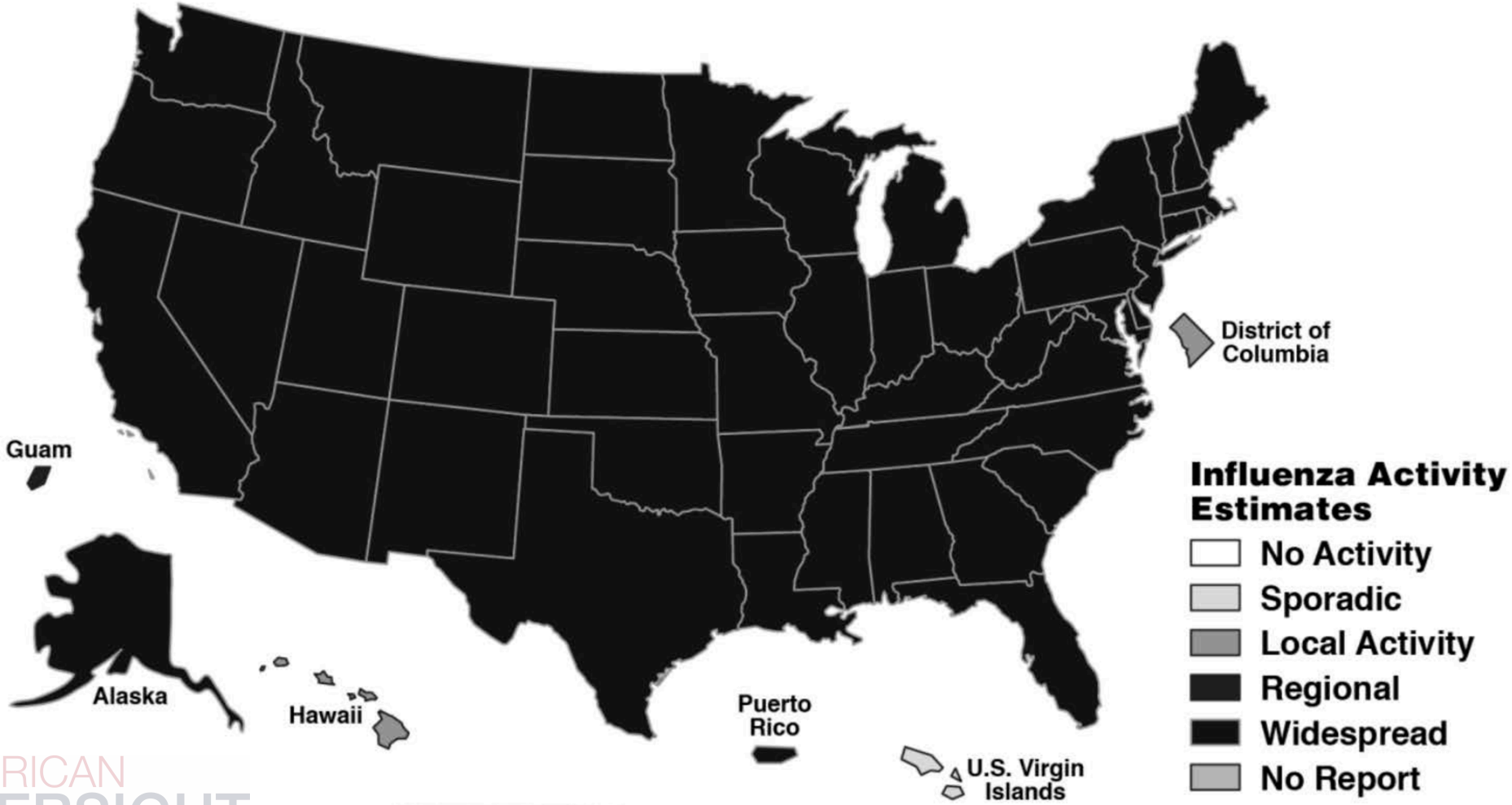
NBC

December 2, 2017

Flu Is Spreading Fast This Year, with a Severe Season Possible

AMERICAN OVERSIGHT

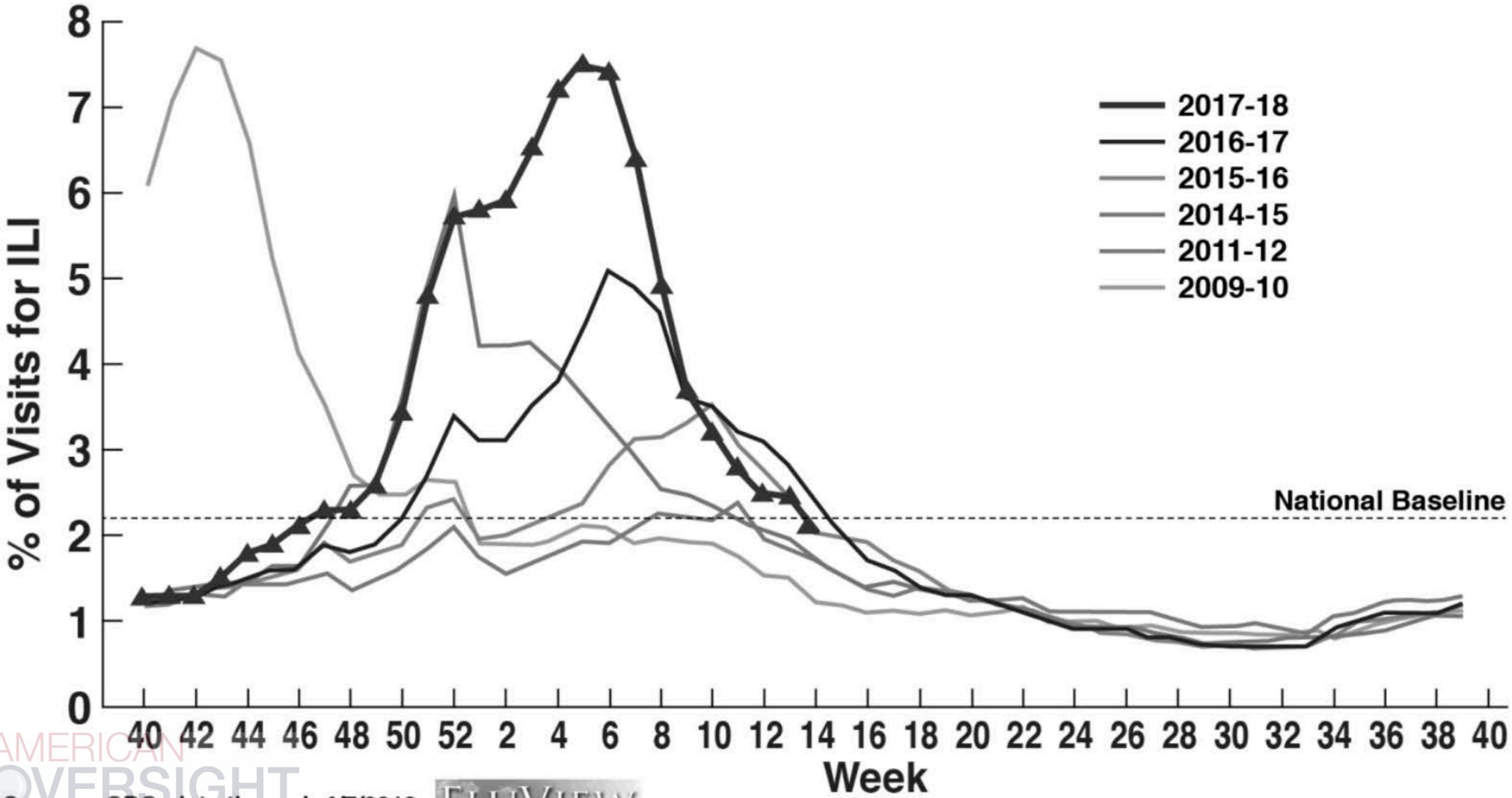
Geographic Spread of Influenza as Assessed by State and Territorial Epidemiologists, January 20, 2018



Source: CDC, week ending 1/20/2018 FLUVIEW

AMERICAN OVERSIGHT

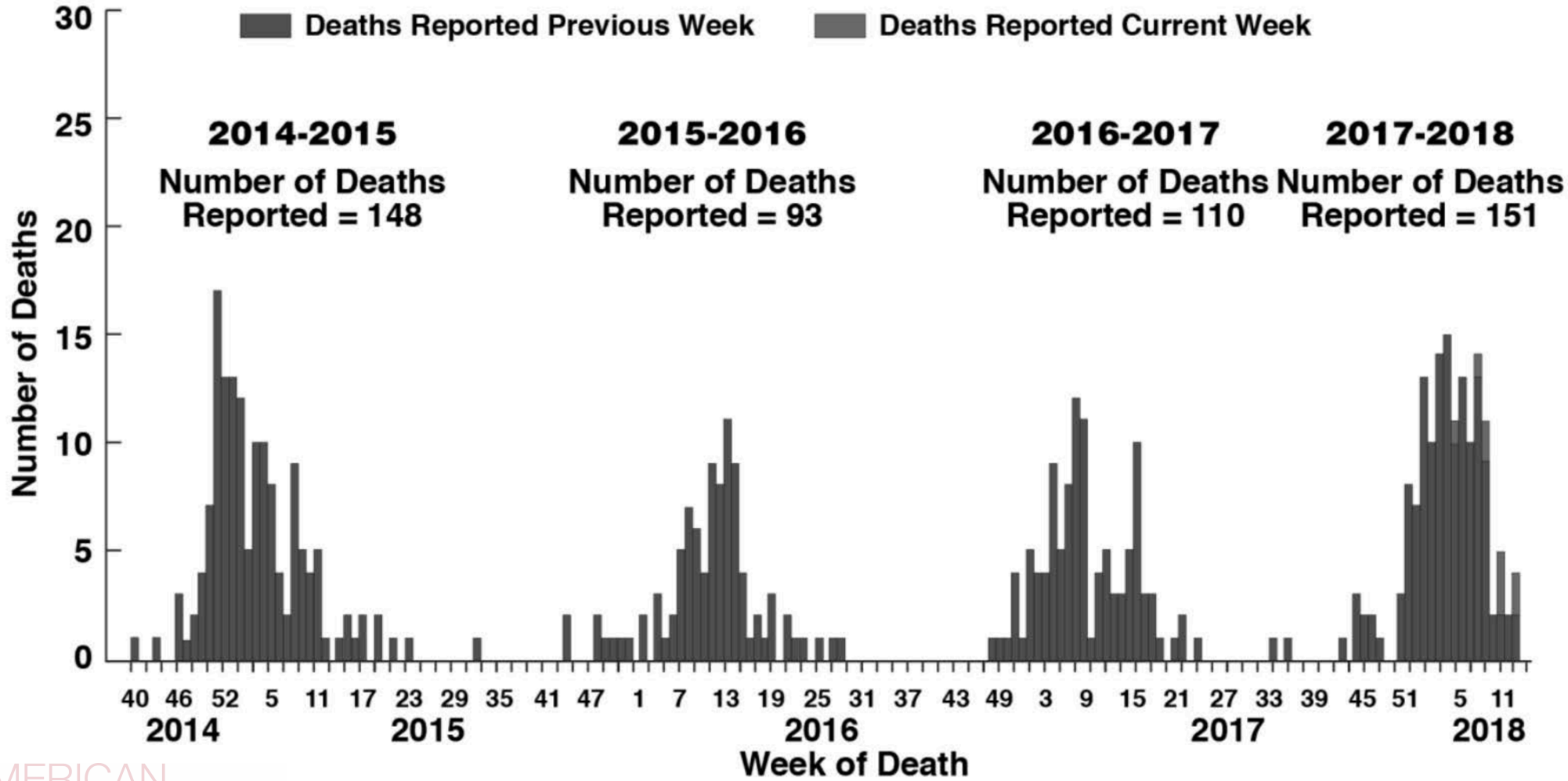
Weekly Reporting of Influenza-Like Illness in the United States by Season



Source: CDC, data through 4/7/2018 FLUVIEW

AMERICAN OVERSIGHT

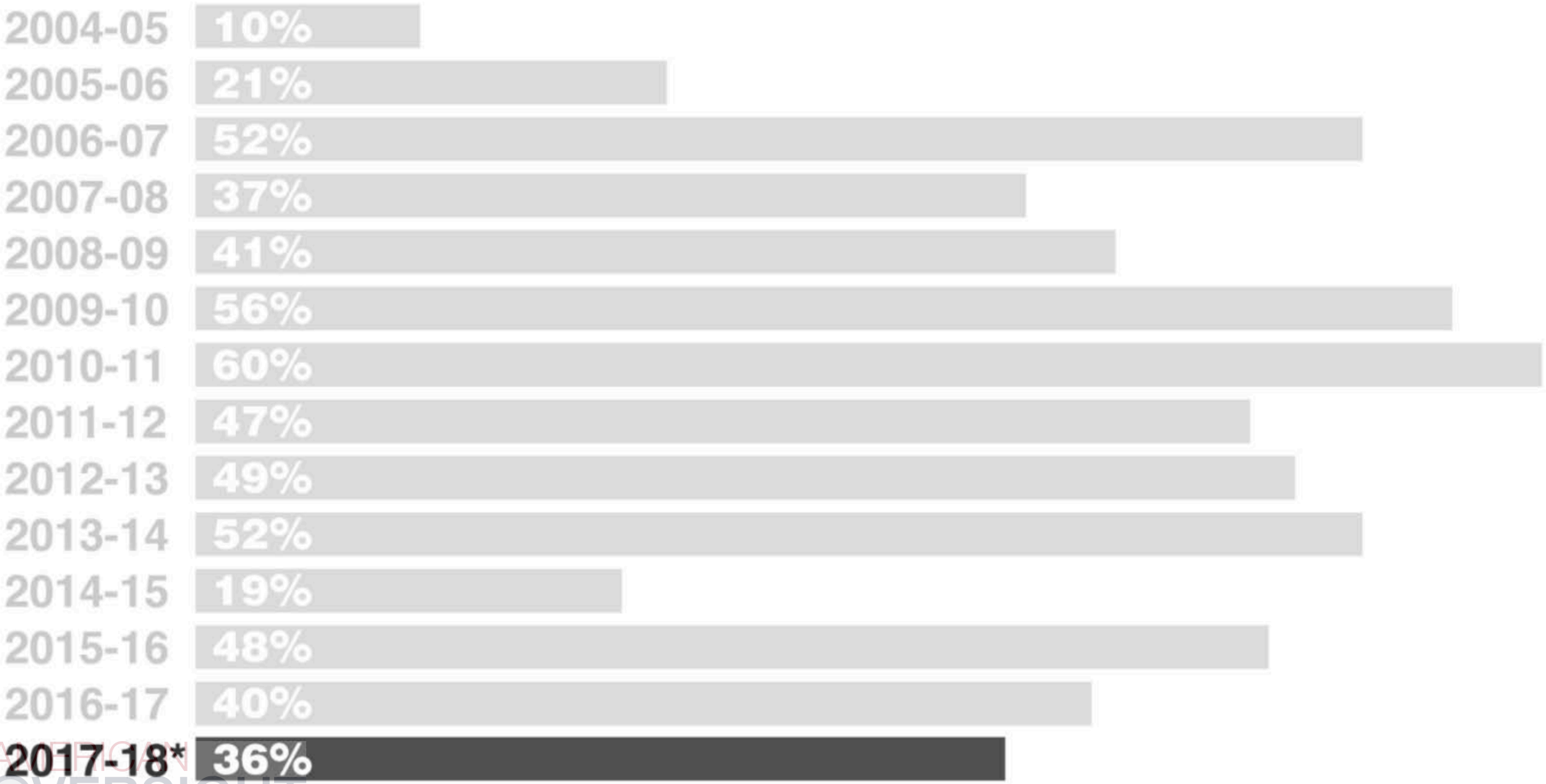
Number of Influenza-Associated Pediatric Deaths by Week of Death



Source: CDC, data through 4/7/2018 FLUVIEW

AMERICAN OVERSIGHT

Adjusted Influenza Vaccine Effectiveness Estimates, United States



*Interim estimate

Source: CDC

AMERICAN OVERSIGHT

Centers for Disease Control and Prevention
MMWR
Morbidity and Mortality Weekly Report
Vol. 67 No. 6 February 16, 2018

Interim Estimates of 2017-18 Seasonal Influenza Vaccine Effectiveness — United States, February 2018

- Adjusted vaccine effectiveness was 36%
  - 25% against influenza A H3N2 viruses
  - 67% against influenza A H1N1 viruses
  - 42% against influenza B viruses

AMERICAN OVERSIGHT

STAT
March 9, 2018

Flu Vaccine Grown Without Eggs Provided Measurably Better Protection This Season, FDA Says

AMERICAN OVERSIGHT

- Current seasonal influenza vaccines are not consistently effective

- Pandemics do occur and response after the fact is not effective

- "Chasing after" potential pandemic outbreaks (pre-pandemic viruses) is costly and ineffective

AMERICAN OVERSIGHT

AMERICAN OVERSIGHT

Beginning of Global H1N1 Influenza as of March, 2009

AMERICAN OVERSIGHT

Source: WHO

Global H1N1 Influenza Cases, as of April 29, 2009

Confirmed or suspected cases

Source: WHO

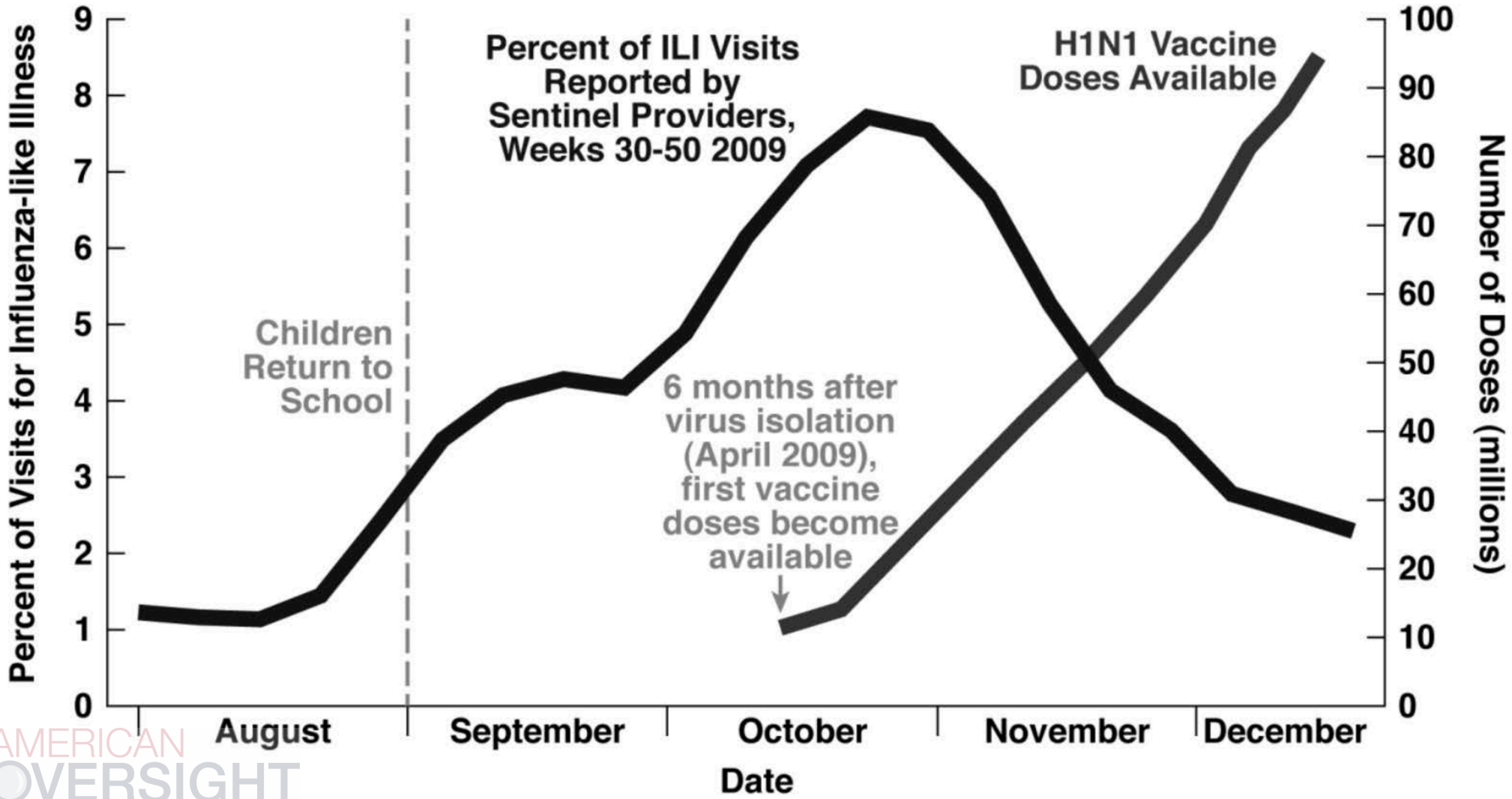
AMERICAN OVERSIGHT

Countries with Confirmed Pandemic (H1N1) 2009 Influenza Cases as of Sept. 30, 2009

AMERICAN OVERSIGHT

Source: WHO

Vaccine Lags Behind 2009 H1N1 Influenza Pandemic



AMERICAN OVERSIGHT

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AMERICAN OVERSIGHT

CDC

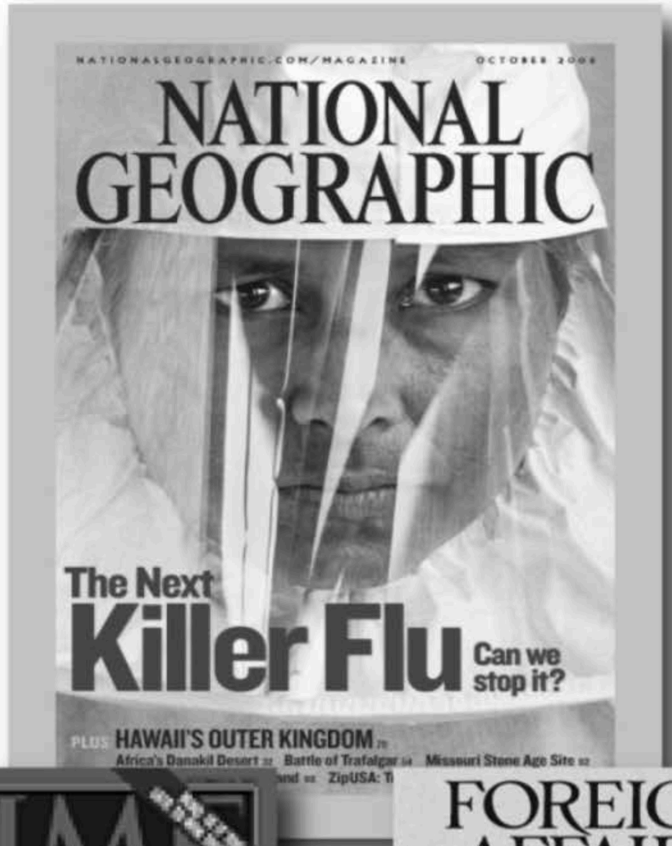
MMWR

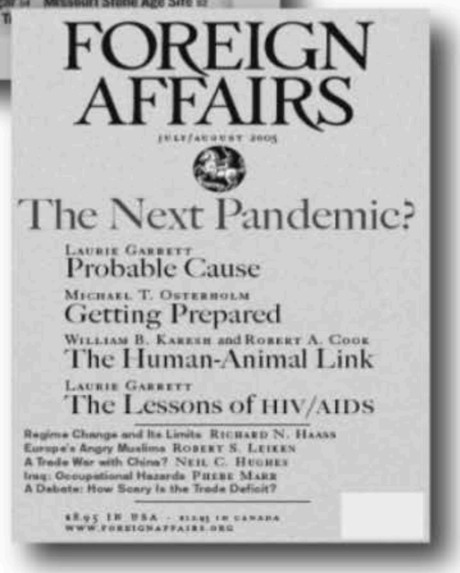
Morbidity and Mortality Weekly Report

Recommendations and Reports December 19, 1997 / Vol. 46 / No. 50

Isolation of Avian Influenza A(H5N1) Viruses from Humans--Hong Kong, May-December 1997

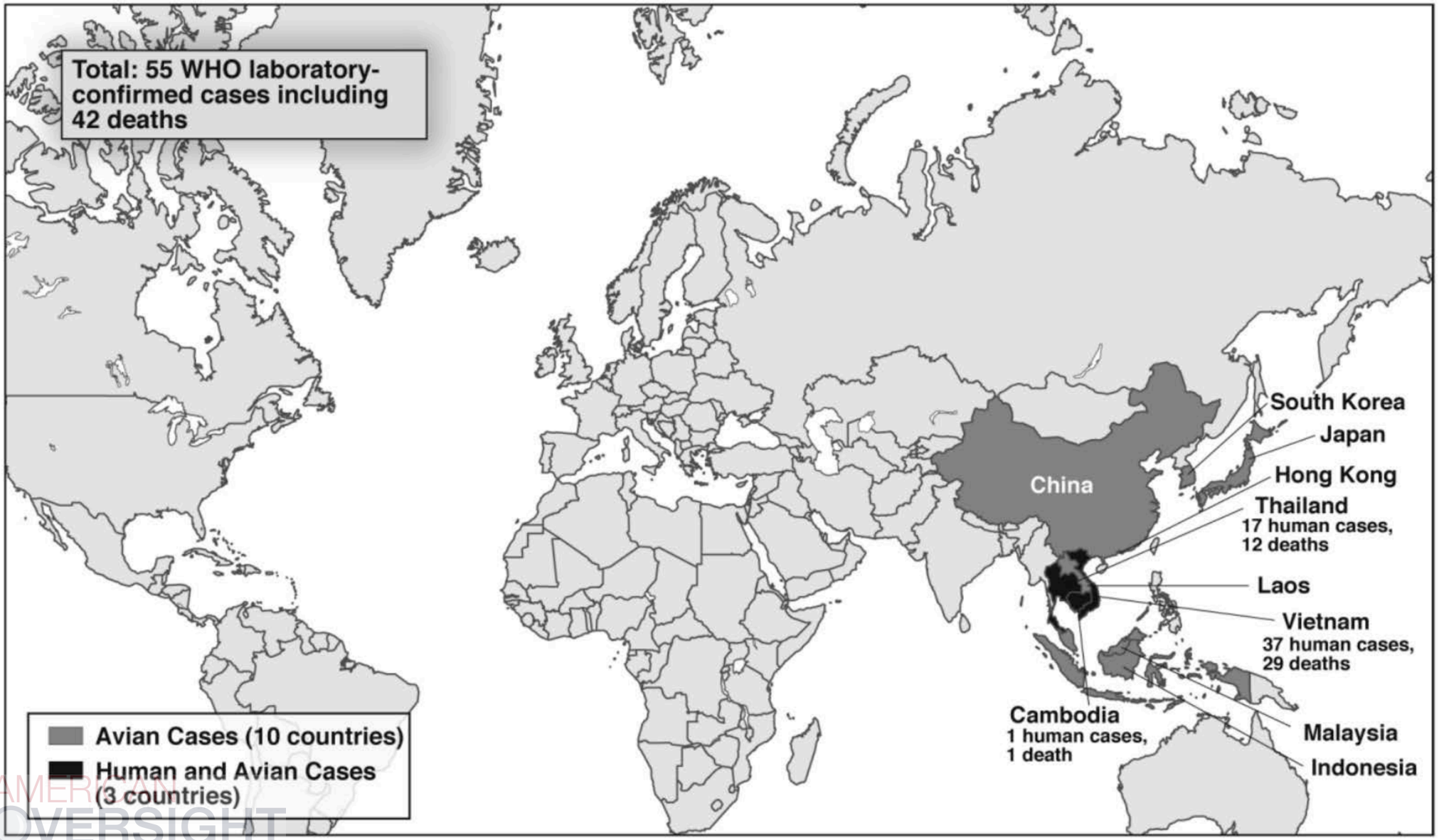
AMERICAN OVERSIGHT





AMERICAN OVERSIGHT

H5N1 Influenza Cases, 2003-2005



Source: WHO 2/2005

AMERICAN OVERSIGHT

HHS News

U.S. Department of Health and Human Services
FOR IMMEDIATE RELEASE
Tuesday, September 21, 2004

www.hhs.gov/news

HHS Orders Avian Flu Vaccine Doses as Preventive Measure

HHS Secretary Tommy G. Thompson announced today the awarding of a contract to Aventis Pasteur Inc. to manufacture and store 2 million doses of avian influenza H5N1 vaccine, an important initial acquisition to better prepare the nation for an influenza pandemic.

The New York Times

November 1, 2005

Bush Calls for $7.1 Billion to Prepare for Bird Flu Threat

President Bush today unveiled a strategy to combat the threat of an avian flu pandemic, calling for $7.1 billion in emergency spending to stockpile reserves of medicines and to press ahead with the development of a new vaccine.

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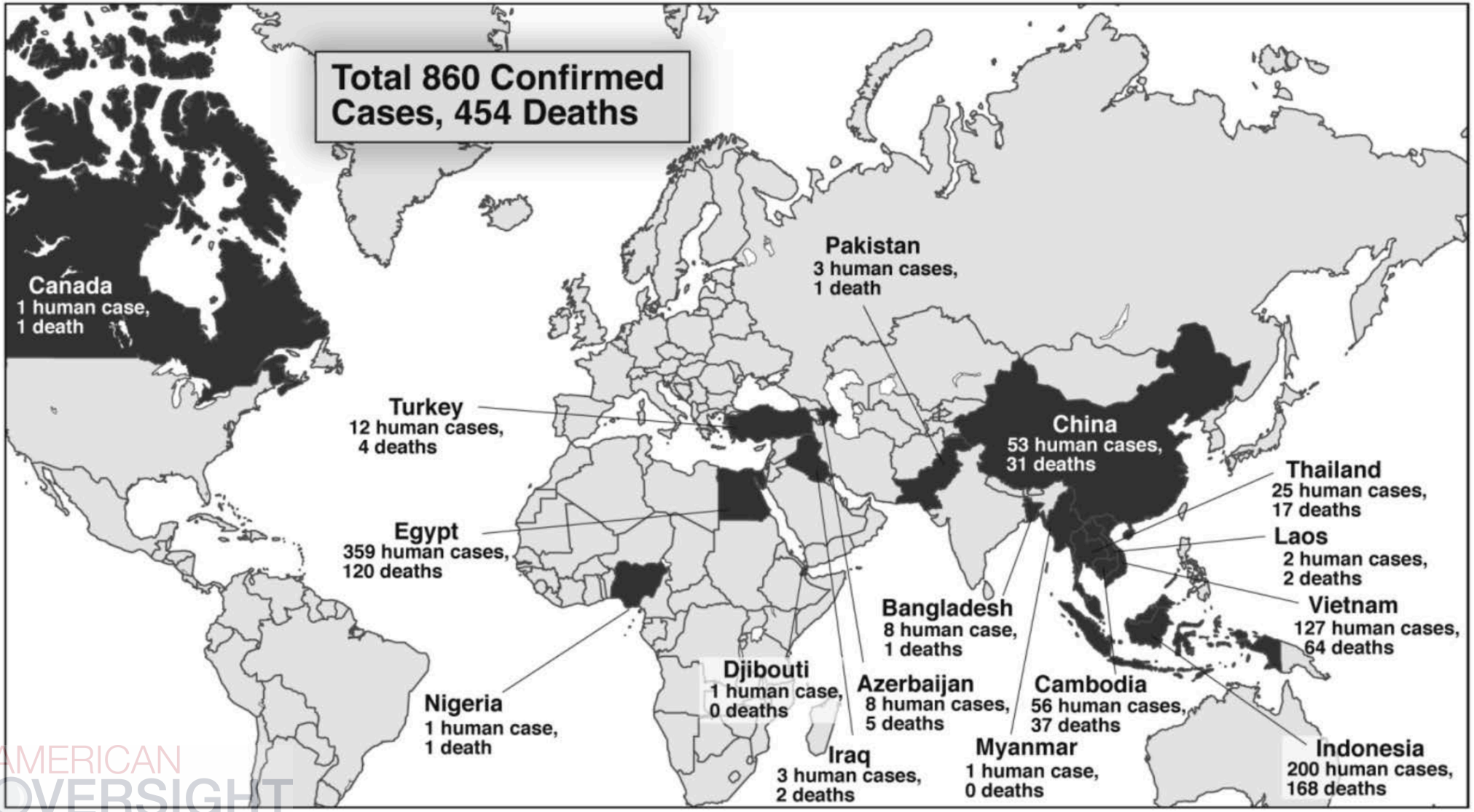
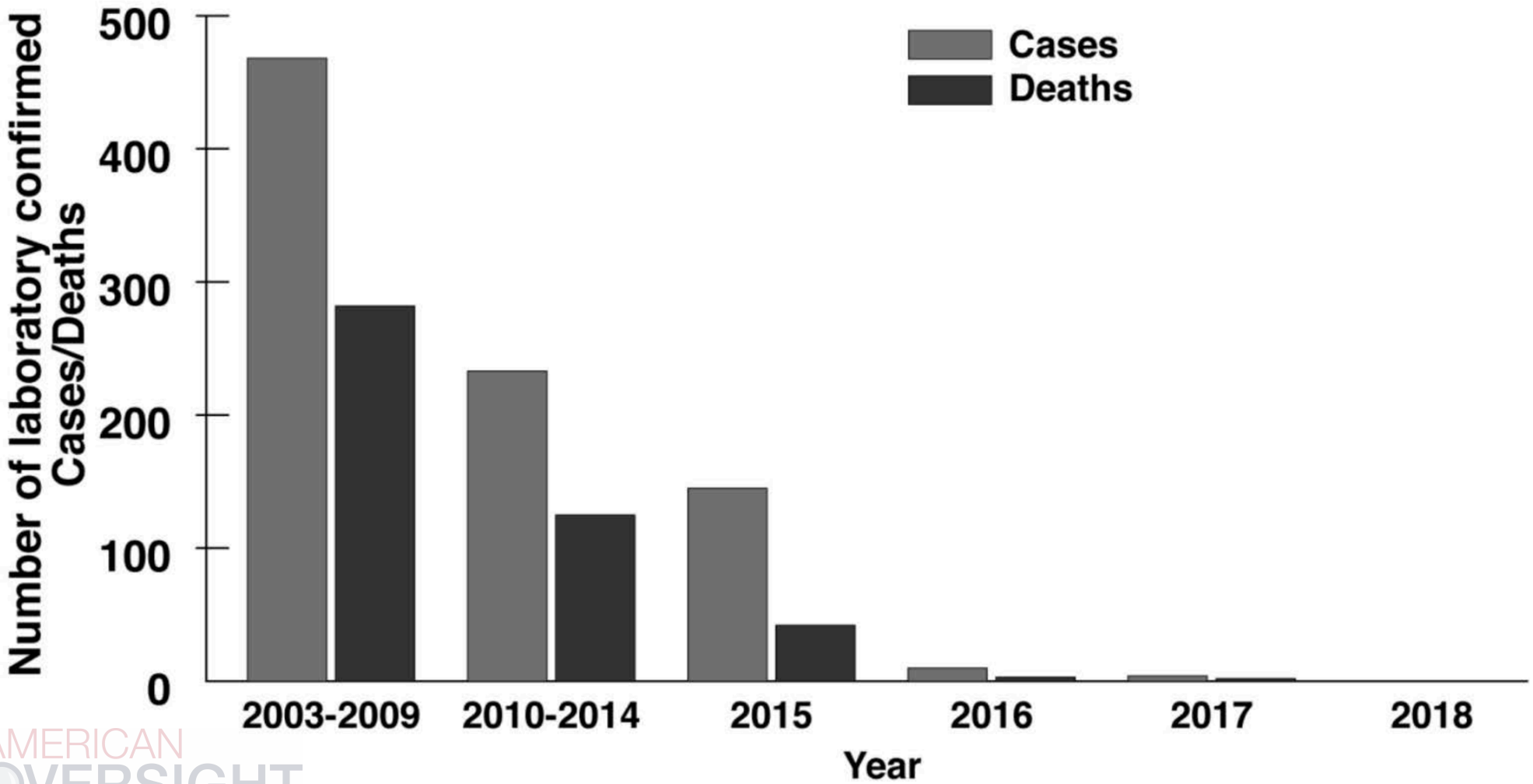
H5N1 Influenza Cases in Humans, 2003-2018



Source: WHO 2018

AMERICAN OVERSIGHT

H5N1 Influenza Cases in Humans, 2003-2018

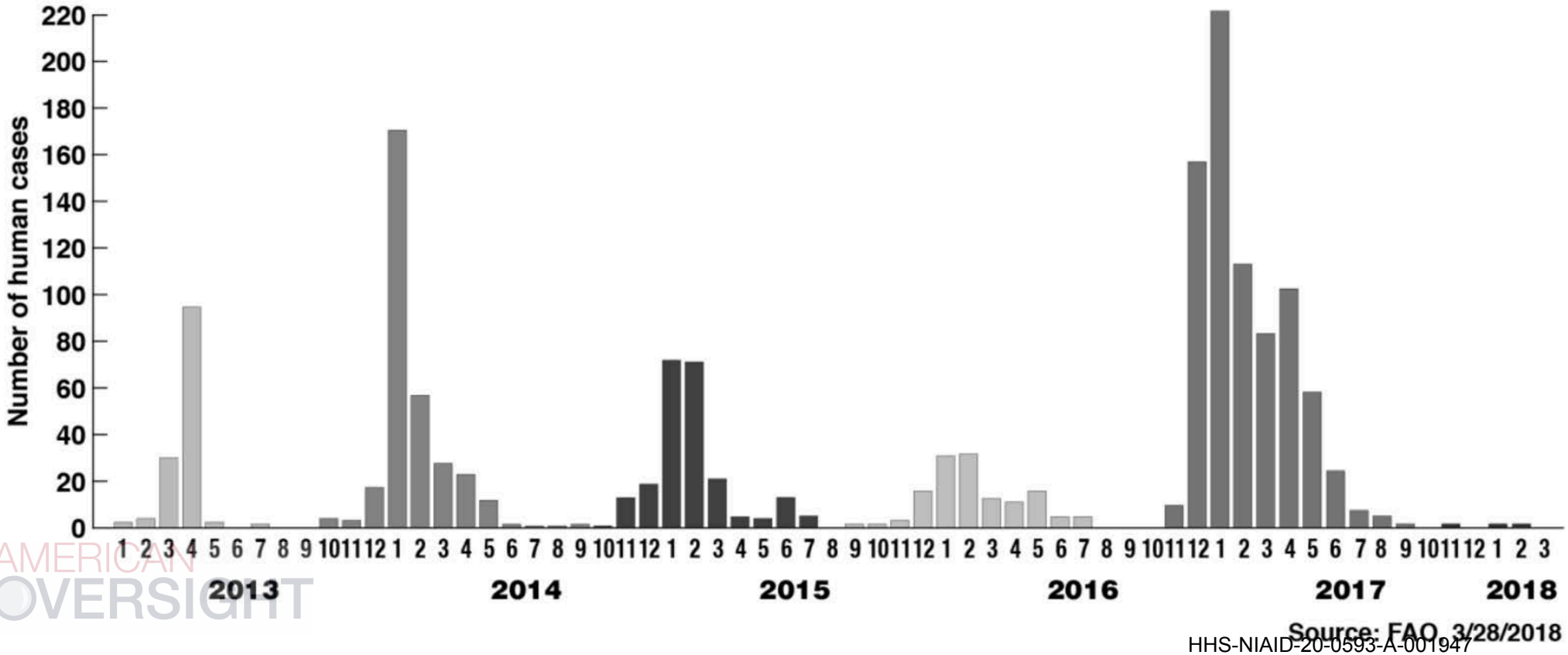


Source: WHO 2018

AMERICAN OVERSIGHT

Waves of Human H7N9 Influenza Infections in China, February 2013-present

- **1,625** confirmed human cases, **622** deaths
- 5th wave: >50% of cumulative cases



AMERICAN OVERSIGHT

STAT

May 1, 2017

As Bird Flu Spreads, US Concludes Its Vaccine Doesn't Provide Adequate Protection

AMERICAN OVERSIGHT

National Institutes of Health
Turning Discovery Into Health

FOR IMMEDIATE RELEASE
Thursday, March 15, 2018

News Release

H7N9 Influenza Vaccine Clinical Trials Begin

- Inactivated 2017 H7N9 influenza vaccine manufactured by Sanofi Pasteur with or without AS03 adjuvant
- Phase 2 trials will enroll up to 420 volunteers ages 19 to 64 years and up to 300 volunteers >65 years of age

AMERICAN OVERSIGHT

Major Challenges in Influenza Vaccinology

- Production of vaccines – Egg-based versus Cell-based versus Recombinant DNA Technology

- Strain-Specificity versus Universal Strain Coverage

AMERICAN OVERSIGHT

Major Challenges in Influenza Vaccinology

- Production of vaccines – Egg-based versus Cell-based versus Recombinant DNA Technology

- Strain-Specificity versus Universal Strain Coverage

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Evolution of Technologies for Influenza Vaccines

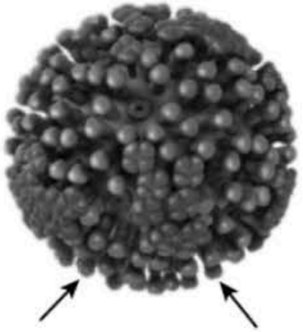
Egg-based → Cell-based → Recombinant DNA Technologies

AMERICAN OVERSIGHT

Vaccine Platform Technologies

Genetic immunization (DNA, RNA)

Viral protein immunization



Flu Virus Hemagglutinin (HA) [H1, H5, H7]

Genetic Immunization (DNA and RNA vaccines)

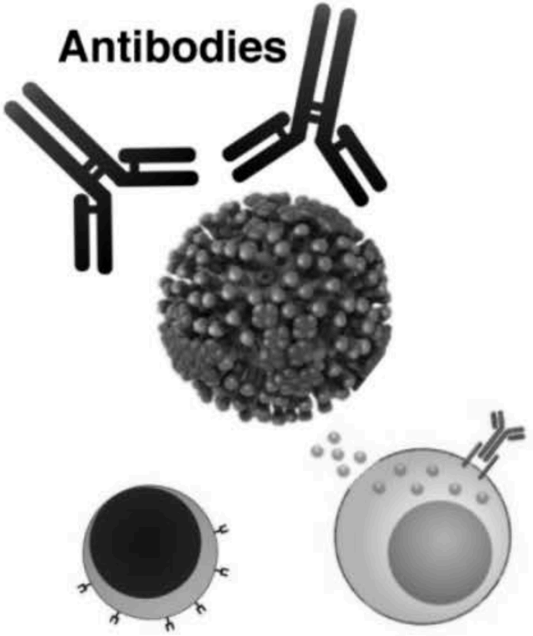
Viral vector (cold virus: adenovirus)

Recombinant protein

Nanoparticles (protein on particle)

Virus-like particle (VLP) (no RNA; non-infectious)

Stimulate the body's immune system to attack the virus



Courtesy of VRC

AMERICAN OVERSIGHT

Major Challenges in Influenza Vaccinology

- Production of vaccines – Egg-based versus Cell-based versus Recombinant DNA Technology

- Strain-Specificity versus Universal Strain Coverage

AMERICAN OVERSIGHT

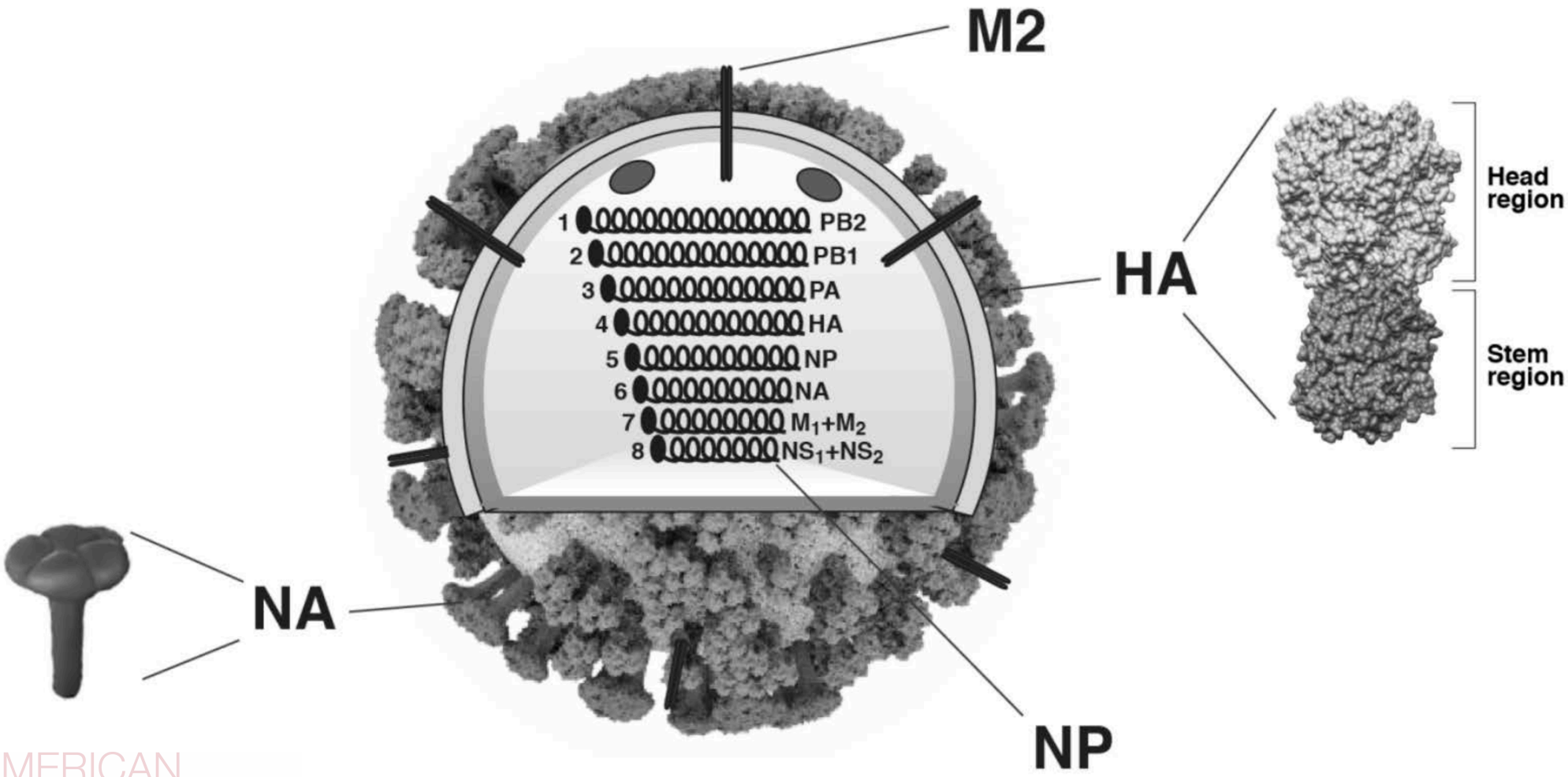
The
New England
Journal of Medicine
Established in 1812 as THE NEW ENGLAND JOURNAL OF MEDICINE AND SURGERY
Volume 378 January 4, 2018 Number 1

Chasing Seasonal Influenza — The Need for a Universal Influenza Vaccine

CI Paules, SG Sullivan, K Subbarao, and AS Fauci

AMERICAN OVERSIGHT

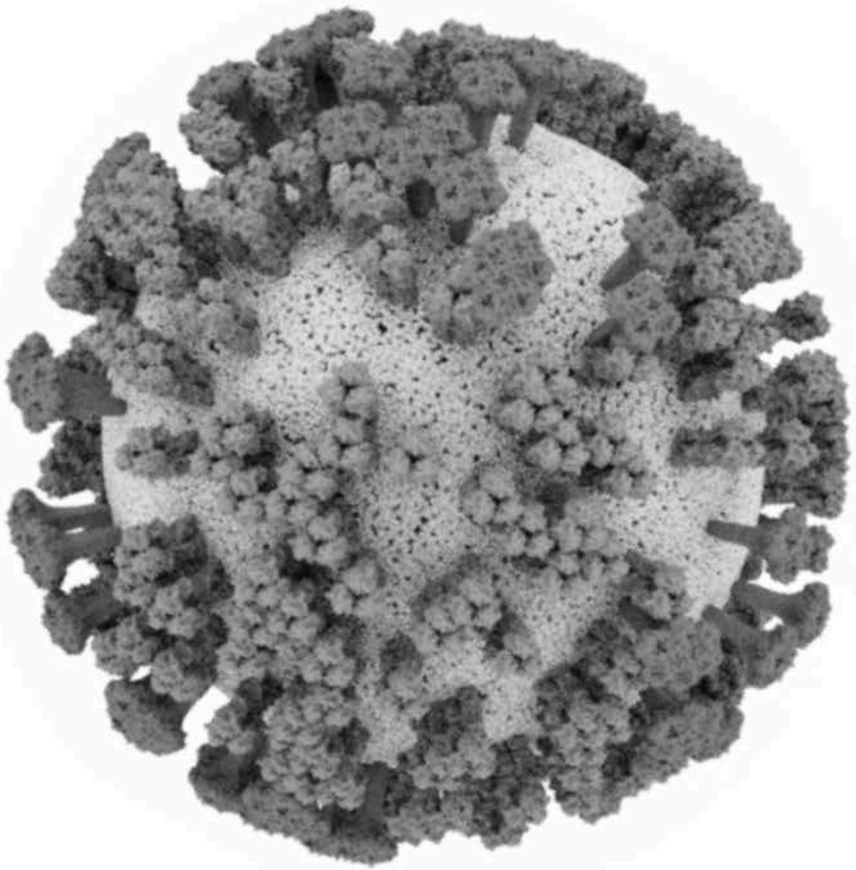
Selected Targets for "Universal" Influenza Vaccines



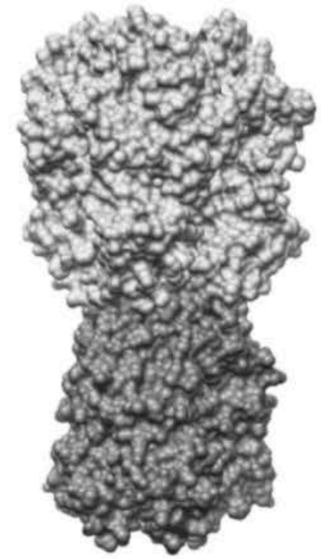
AMERICAN OVERSIGHT

Courtesy of VRC

Hemagglutinin Protein: Major Target of Influenza Vaccines



Influenza Virus

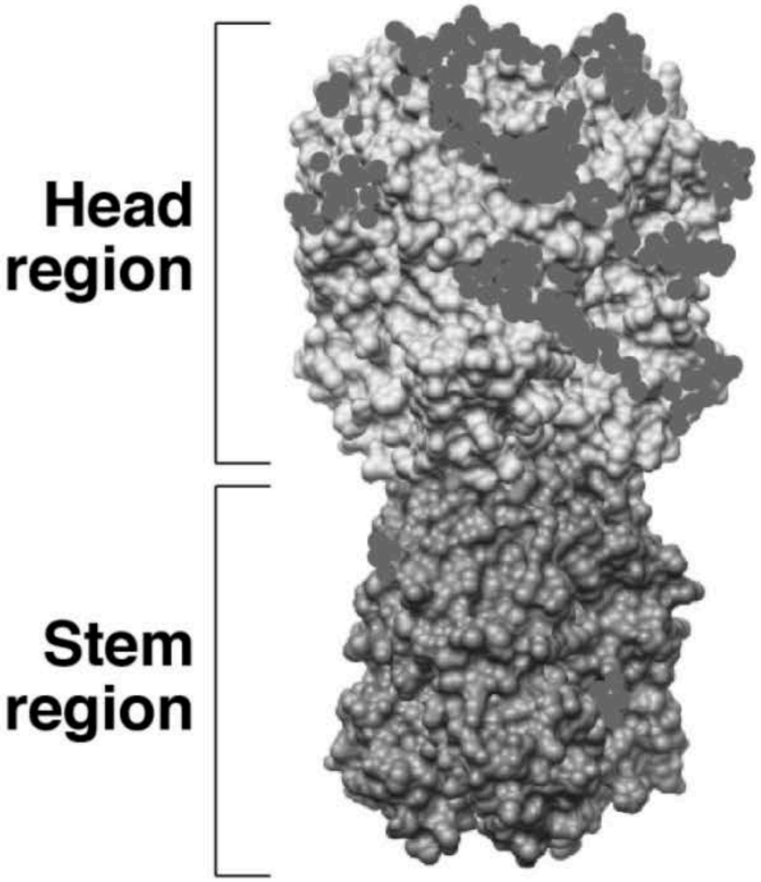


Hemagglutinin (HA) Protein

AMERICAN OVERSIGHT

Courtesy of VRC

Influenza A Hemagglutinin (HA)



Head region

- Target of current influenza vaccines
- Differs among influenza strains
- Many mutations (●) each season

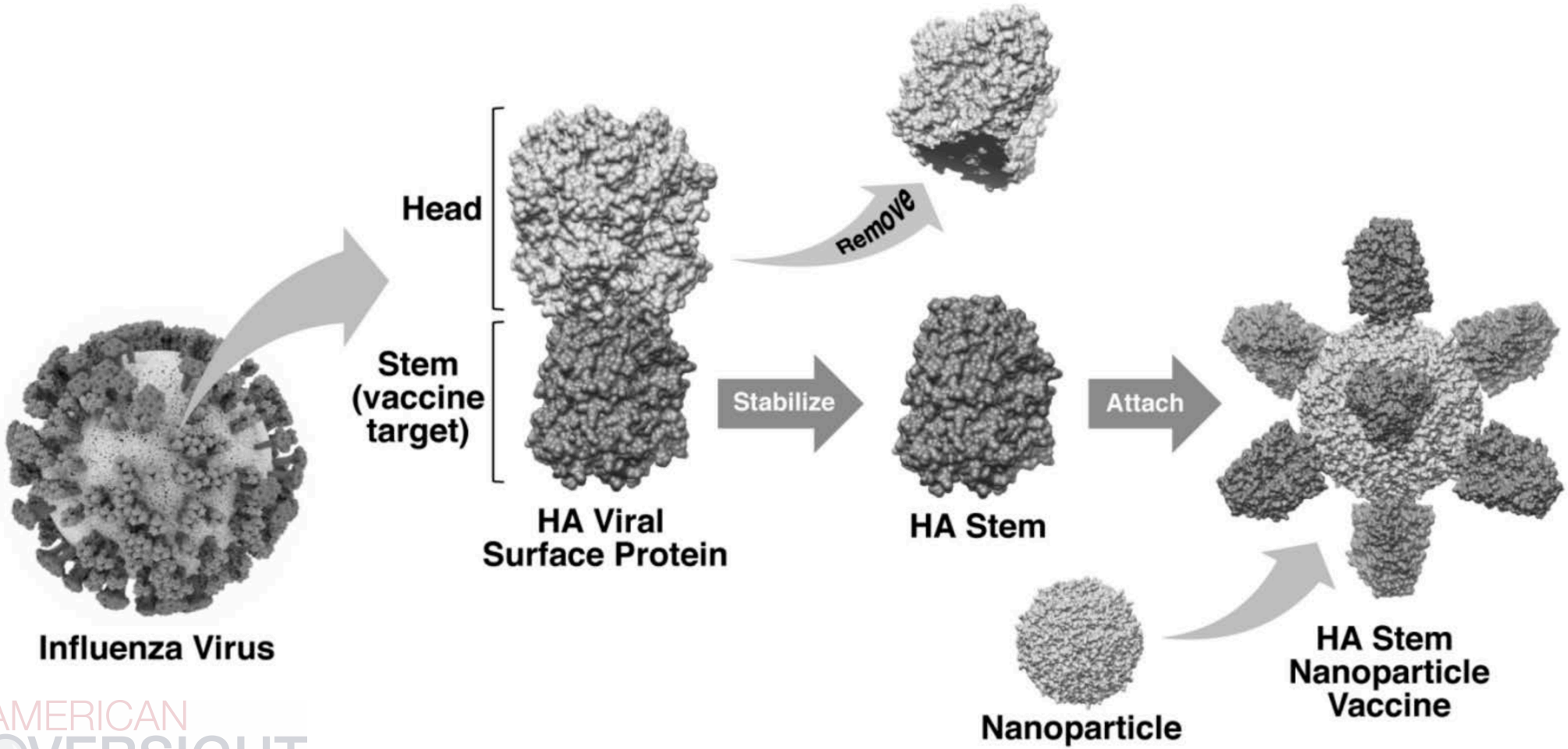
Stem region

- Target of universal influenza vaccines
- Similar among influenza strains
- Few mutations each season

AMERICAN OVERSIGHT

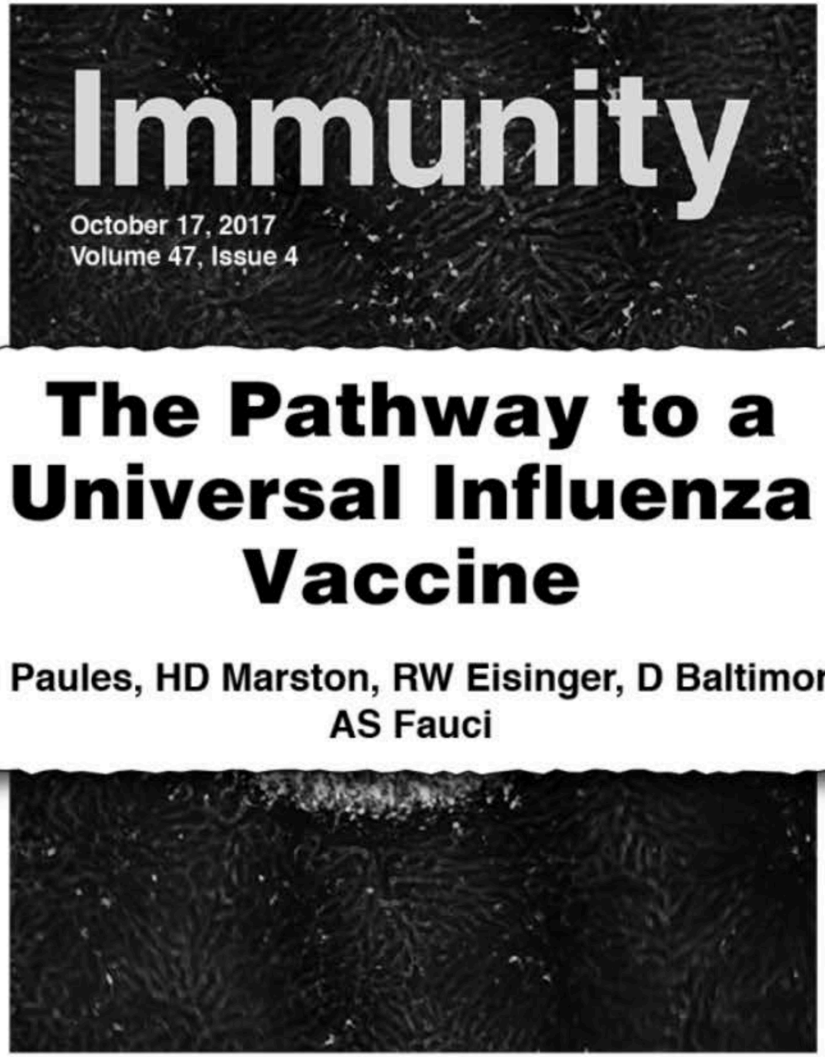
Courtesy of VRC

Representative Approach to the Development of a Universal Influenza Vaccine



Courtesy of VRC

AMERICAN OVERSIGHT



The Pathway to a Universal Influenza Vaccine

CI Paules, HD Marston, RW Eisinger, D Baltimore, AS Fauci

AMERICAN OVERSIGHT

Published online
February 28, 2018

The Journal of Infectious Diseases

IDSA Infectious Diseases Society of America

hivma hiv medicine association

A Universal Influenza Vaccine: The Strategic Plan for the National Institute of Allergy and Infectious Diseases

EJ Erbelding, D Post, E Stemmy, PC Roberts, A Deckhut Augustine, S Ferguson, CI Paules, BS Graham, AS Fauci

AMERICAN OVERSIGHT

- **Three key research areas:**
  - Improve understanding of transmission, natural history, and pathogenesis of influenza infection
  - Precise characterization of influenza immunity and correlates of immune protection
  - Support rational design of universal influenza vaccines

NIH IC Directors' Meeting
Building 1, Room 151
Thursday, April 26, 2018
8:30 AM-10:00 AM

Agenda

8:30 AM	Opening Remarks Larry Tabak
8:45 AM	Managing Bias in Official Duty Activities Mike Lauer
9:15 AM	2017-2018 Flu Season/Need for a Universal Flu Vaccine Tony Fauci

AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Sat, 21 Apr 2018 19:53:41 +0000
To: Grady, Christine (NIH/CC/BEP) [E]
Subject: RE: Read this

Here is the quote from the Times article. It refers to Seema's article in Science:

> "It's really insulting," said Dr. Stephen Whitehead, a vaccine researcher at the N.I.H., said of the panel's decision. "We've been painted as mad scientists who do horrible studies on human beings. But we're on top of all these risks."

Human-challenge studies are only conducted at a small number of institutions in the United States. The trials have been used to test vaccines or treatments for dengue, malaria, influenza and norovirus, among other diseases.

Natural Zika outbreaks are becoming too sporadic to test vaccines, so until the next large outbreak, a human-challenge study may be the only way for vaccine developers to proceed.

"This ethics consultation was debilitating for the whole community," said Dr. Anna Durbin, a research clinician at Johns Hopkins University who collaborated on the study's design. "It really slammed the door on progress."

From: Grady, Christine (NIH/CC/BEP) [E]
Sent: Saturday, April 21, 2018 3:39 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: Re: Read this

I can't open the link

Sent from my iPad

On Apr 21, 2018, at 1:41 PM, Fauci, Anthony (NIH/NIAID) [E] (b)(6) wrote:

Cliff/Steve/Courtney:
OK. Read the quotes from Steve Whitehead and Anna Durbin in the New York Times (see link below) and let us discuss.

AMERICAN OVERSIGHT

https://www.nytimes.com/2018/04/20/health/zika-study-ethics.html

Anthony S. Fauci, MD
Director
National Institute of Allergy and Infectious Diseases
Building 31, Room 7A-03
31 Center Drive, MSC 2520
National Institutes of Health
Bethesda, MD 20892-2520
Phone: (b)(6)
FAX: (301) 496-4409
E-mail: (b)(6)
The information in this e-mail and any of its attachments is confidential and may contain sensitive information. It should not be used by anyone who is not the original intended recipient. If you have received this e-mail in error please inform the sender and delete it from your mailbox or any other storage devices. The National Institute of Allergy and Infectious Diseases (NIAID) shall not accept liability for any statements made that are the sender's own and not expressly made on behalf of the NIAID by one of its representatives.

AMERICAN OVERSIGHT

From: Fauci, Anthony (NIH/NIAID) [E]
Sent: Sat, 21 Apr 2018 16:41:31 +0000
To: Luc Debruyne
Cc: Marston, Hilary (NIH/NIAID) [E];Paules, Catharine (NIH/NIAID) [E]
Subject: RE: Collaboration NIAID / GSK

Luc:
Thank you for your kind note. We here at NIAID also look forward to continued collaboration with you in this important area of research and development.
Best regards,
Tony

From: Luc Debruyne (b)(6)
Sent: Thursday, April 19, 2018 12:21 PM
To: Fauci, Anthony (NIH/NIAID) [E] (b)(6)
Subject: Collaboration NIAID / GSK

Dear Dr Fauci
Dear Tony

I was pleased to hear of the strong congressional support for NIAID to continue breaking ground on novel vaccine technologies to prevent and mitigate pandemic influenza and other emerging infectious diseases. I am encouraged by Congress' recognition that we must be vigilant and proactive in our efforts to counter infectious disease threats, whether they be endemic, naturally emerging, or man-made. It is also critical that Congress recognizes the essential role that the NIH plays to advance science to address the world's most pressing public health concerns.

I also read with great interest your recent article in JAMA, "Novel Vaccine Technologies: Essential Components of an Adequate Response to Emerging Viral Diseases." You and your colleagues are right. If we fail to harness the potential of platform technologies and other advances in vaccinology now, we will not be able to adequately respond to the next emerging infectious disease threat. As we discussed in our most recent meeting, GSK is investing in the next generation of vaccine technologies and infrastructure so that we can contribute to the next response when vaccine manufacturers are called upon to act. However, as you know, it is imperative that the US government also invest in these efforts if they are ever to become a reality.

We look forward to continued collaboration with NIAID and its partner agencies in the coming years to ensure that these capabilities are fully realized and ready when that moment inevitably comes.

I wish you well and I hope that we may meet again soon.

Best wishes

Luc

Luc Debruyne

AMERICAN OVERSIGHT

President Global Vaccines

GSK
20 Avenue Fleming, 1300 Wavre, Belgium
Email (b)(6)
Tel (b)(6)

gsk.com | Twitter | YouTube | Facebook | Flickr

GSK monitors email communications sent to and from GSK in order to protect GSK, our employees, customers, suppliers and business partners, from cyber threats and loss of GSK Information. GSK monitoring is conducted with appropriate confidentiality controls and in accordance with local laws and after appropriate consultation.

AMERICAN OVERSIGHT

From: Eisinger, Robert (NIH/NIAID) [E]
Sent: Fri, 20 Apr 2018 22:11:16 +0000
To: Fauci, Anthony (NIH/NIAID) [E]
Cc: Doepel, Laurie (NIH/NIAID) [E]
Subject: FW: Lancet Commission final pre-submission draft Review
Attachments: TB Executive summary_041918 rev be ld.docx, Section 0_041918 rev be ld.docx, Section 1_041918 rev be ld.docx, Section 2_041918 rev be ld.docx

Please find in the attachments the:

- Executive Summary (Recommendations/Priorities)
- Section 0 (Report Roadmap/Overview)
- Section 1 (Strategies to reduce TB mortality)
- Section 2 (Research) with modifications from Laurie and me.

Due to the size of the files, I will provide the additional sections (Section 3 – Macroeconomics; Section 4 - Accountability; and Panels – Background on Specific Topics) with our revisions in the next email.

I am on your schedule for Monday morning to discuss the entire document. After our meeting, I will draft an email for you to send to Eric that will highlight the key modifications/issues to the Report.

Thanks
Bob

From: Reid, Michael (b)(6)
Sent: Friday, April 20, 2018 3:14 AM
To: Goosby, Eric <Eric.Goosby@ucsf.edu>
Cc: Dean Jamison (b)(6) Soumya Swaminathan (b)(6)
Subject: Re: Lancet Commission final pre-submission draft Review

Dear Commissioners,

Thank you to all those of you that have contributed to revising the report and responding to the external reviewer comments. In particular, thanks to the teams from NIH and FIND for their help pulling pieces together!

Please find attached a zip file containing the current draft [minus a few pending analyses]. The full report – in 6 sections – can also be downloaded:

From Box: (b)(6)

From Dropbox: (b)(6)

In order to ensure that we have the best chance of getting the report published in time for the HLM, The Lancet have requested that we formally submit by **April 27th**. I fully appreciate that this means everyone has a short time to review and respond. Nonetheless, we would be grateful for your comments/edits by **April 24** if possible.

AMERICAN OVERSIGHT

In particular, please review the executive summary, in particular the 'vision statement' and the more concise recommendations (5 rather than 10), which align with the HLM 'key asks'.

Eric and I welcome your feedback via email. If possible, share any substantive recommendations/comments in the email, rather than track changes to the documents, to ensure we don't miss any major comments. However, feel free to use track changes if that is your preference. I can also be available most of next week (April 23-26) by 'phone or Skype to discuss questions/clarifications/concerns.

Finally, several of you partnered with non-commissioners to complete writing/analytic products – If you feel these colleagues should be considered as co-authors on the publication, *and you haven't already reached to me to discuss*, please let me know.

Thank you all for your continued support and collaboration in this effort and the important roles you each play in 'building a TB-free world.'

Best regards,

Mike

Lancet Commission on TB, Secretariat

From: "Reid, Michael" (b)(6)
Date: Sunday, April 15, 2018 at 06:22
To: Eric Goosby (b)(6)
Subject: Re: Lancet Commission Conf calls - week of April 16-20

Dear Commissioners,

Apologies for the delay in failing to circle back on this email from David sooner. Unfortunately, we were unable to find any date/time that worked for more than 2 or 3 people. With this in mind, Eric and I have decided to forgo a conference call for all Commissioners.

We are aiming to formally submit a revised draft of the Commission, as well as responses to all the external reviewers' comments, to The Lancet by **April 27**. In advance of that date, I intend to share a pre-submission draft of the full report to all Commissioners this coming Wed/Thurs [**April 18/19**]. I kindly ask that you all take time to review and make edits/comments – sending back any comments/edits no later than April 23.

I can personally be available for one-on-one calls on April 23 and 24 for anyone that wants to give feedback over the 'phone. Please email me individually if you would like to connect on either day.

Thanks for all of your input, particularly those of you that have been re-writing or reviewing subsections of the report over the last 2 weeks.

AMERICAN OVERSIGHT

Kind regards,

Mike Reid
(on behalf of the Commission Secretariat)

From: David McKey (b)(6)
Date: Wednesday, April 4, 2018 at 09:52
To: David McKey (b)(6)
Cc: "Reid, Michael" (b)(6)
Subject: Lancet Commission Conf calls - week of April 16-20

Dear Commissioners,
As a follow-up to our recent meeting in New Delhi, it was agreed that we would arrange for two conference calls the week of April 16-20 to give an update on the progress and discuss any last minute details before the report is submitted the end of April.

Please find the link for a doodle poll indicating possible times for a call. Please select the two dates and times that work the best for you.
https://doodle.com/poll/nbt5qfuq9x96ddpw

If you plan on submitting expenses for reimbursement, please send as soon as possible. As an FYI, I will be out on holiday 10-22 April. Expenses not received before 6 April will be submitted upon my return to the office.

And, attached is the picture from our meeting.

Thank you,
David

David McKey
Office of the UN Secretary-General's Special Envoy on Tuberculosis
Global Health Delivery & Diplomacy
Institute for Global Health Sciences | UCSF
550 16th Street | 3rd Floor | Box 1224
San Francisco, California 94158

(b)(6)

AMERICAN OVERSIGHT

(b)(6)

UCSF Institute for Global Health Sciences

AMERICAN OVERSIGHT

EXECUTIVE SUMMARY: LANCET COMMISSION ON TB

The Lancet Commission on Tuberculosis: Building a TB-Free World

Executive Summary

April 19, 2018

Vision: *With bold leadership, smart investments based on sound science, and a shared responsibility, we will end TB within a generation*

Vision: *Any country that commits resources to a tightly focused TB control agenda -responding effectively to known cases and finding and preventing cases in high risk groups -can end TB in a generation*

Commented [MOU1]: Please help decide which of these you would go for…

Alternatively, please suggest an alternative?

Commented [ER([2R2]: Propose – Vision: With bold leadership, accelerated research and development, implementation of evidence-based strategies, and a shared responsibility, we will end TB within a generation.

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EXECUTIVE SUMMARY: LANCET COMMISSION ON TB

The Lancet Commission on Tuberculosis: Building a TB-Free World

Executive Summary

For too long tuberculosis (TB) has been the "forgotten" disease, relegated to the back seat behind other major infectious diseases~~killers, including HIV/AIDS and malaria~~. Its second-class status has persisted even though today TB kills more people each year than any other infectious disease—and tragically, although we have in hand proven tools to prevent, treat, and cure it.

In 2016, seven countries accounted for 64% of the estimated 10.4 million new TB cases worldwide, and 1.74 million people with TB died from the disease, including ~~; another~~ 400,000 people ~~who died~~ with HIV ~~were coinfected with TB at the time of their deaths~~. Unless mortality rates decline substantially, another 24 million people will die of TB by 2030. Even more alarming, almost 2 billion people are latently infected with *Mycobacterium tuberculosis* and at risk of developing active TB disease at any time.

Why are we still not making significant progress against this ancient foe? First, many people with TB live in poverty, an essentially "voiceless" constituency when it comes to advocacy and even more importantly, access to diagnosis, treatment, and care. ~~Often t~~The poor often ~~cannot~~ have no access to high-quality TB services, ~~or they~~ or face ~~crippling~~ ruinous medical expenses that preclude~~prevent~~ them from getting the care they need. Consequently, up to 40% of people with TB disease are not being diagnosed or notified to national TB programs. Second, strategies to identify TB cases in high-risk populations, such as people living with HIV, migrants, and prisoners, are fragmented~~piecemeal~~ or non-existent. Despite compelling evidence that TB preventive therapy can save lives, ~~is life-saving treatment,~~ especially those of ~~for~~ people living with HIV and household contacts of ~~those~~people suffering from TB, prophylactic therapy~~it~~ is rarely offered in high-burden countries. Third, TB research and development (R&D) is chronically under-funded. ~~The resulting inadequate pace of TB R&D has helped fuel an epidemic of drug-resistant forms of TB (DR TB).~~ Unless urgent steps are taken to significantly increase R&D funding to enable the development of new and more patient-friendly treatment strategies, as well as novel diagnostics and vaccines, DR TB ~~will~~is projected to cause 2.5 million deaths a year by 2050. The global health security implications of uncontrolled DR TB cannot be overstated. It also is critical to support research on TB and HIV coinfection. Finally, recent global efforts to end TB ~~since 2015~~ have

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Commented [ER([4]: The major reason for DR-TB is failure to adhere to and complete treatment regimens.

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been undermined by a lack of both political will and financial investments. Despite economic analysis, commissioned for this report, demonstrating that the benefits from preventing TB deaths will exceed costs by a factor of at least ten, current funding efforts fall woefully short of what is required.

Undeniably, the global community now finds itself at a tipping point with TB control. While many of the challenges that plague TB control today have existed for generations, only recently have we witnessed the tide beginning to turn. Increasingly, data-driven science is and must continue guiding our efforts to end TB. There is now growing recognition that by immediately scaling-up our use of evidence-based strategies~~proven strategies~~ as well as increasing our investment in TB R&D to bring transformative new tools quickly in hand, we can accelerate progress and bend the curve on this deadliest of infectious diseases.

In June 2017, the Lancet Commission on TB—a diverse group including experts in TB, public health, and development, as well as TB survivors, advocates, and political leaders—was established to investigate how current and future TB elimination efforts could meet the ambitious targets outlined in the WHO's End TB strategy. Working under the assumption that with targeted strategies, smart investments based on sound science, and a shared responsibility, we can end TB within a generation, the Commission set out to answer the question: *How should TB high-burden countries and their development partners target their future investments to ensure that ending TB is achievable?*

This Commission asserts that to invest wisely to save more lives, we must focus TB investments in the five priority areas outlined in Panel A.

Seizing this moment

Fortunately, while the challenges are many, hope is on the horizon. We have rapid and more sensitive diagnostic tools and the promise of a few less toxic, more potent TB treatments in the pipeline. Programmatic innovations, new health technologies, sustained global economic growth, increased commitment to achieving Universal Health Coverage (UHC), and growing political momentum to definitively address TB all make ending the TB epidemic more achievable than ever before.

In September 2018, a historic High-Level Meeting of all United Nations (UN) member states as part of the UN General Assembly will convene to make defeating TB a global political priority. After years of neglect, public health and political leaders worldwide recognize that ending TB is within our grasp. To

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capitalize on this critical moment in time~~juncture~~, we must model lessons learned and implement strategies that ~~can~~will have the most~~aximal~~ impact. The next phase of the global TB response ~~will~~ demands an inclusive, comprehensive response within a broader sustainable development agenda.

Bold, comprehensive strategies

No one-size-fits-all approach can be successful. Ending the TB epidemic will require countries to tailor their TB programs using a variety of new strategies needed to address their different epidemiologic and programmatic challenges. Nonetheless, the ~~first~~ priority in all contexts must be to respond comprehensively and effectively to all known cases, ensuring that everyone with TB has~~ve~~ access to high- quality diagnostic and treatment services, and to patient-centered resources to support them throughout the care cascade. In addition, we must *detect*, *treat*, and *prevent* TB in family members and close contacts of those with TB. In India, the country with the highest TB burden, strategies that improve the quality of care for those presenting in the private sector will be important; modeling analysis demonstrates that by optimizing private sector engagement, we will avert 30 million deaths between now and 2045 (Figure 1A). In high DR TB burden countries, a key priority is access to drug susceptibility testing (DST) and second-line drugs. Our modeling demonstrates that in a country like Moldova, where more than 25 percent of all *Mtb*~~TB~~ is drug-resistant, improving access to DST and second-line drugs would reduce DR TB mortality by 44% in the coming 30 years (Figure 1B).

Second, we must actively reach out to high-risk populations, such as migrants, prisoners, and people living with HIV or diabetes, both to detect TB cases and to prevent ongoing transmission among those at high risk. Targeting active case finding and preventive therapy to these high-risk populations ~~will be~~ is essential to controlling the epidemic. In Kenya, for example, where rates of HIV/TB co-infection are high, scaling up access to both antiretroviral therapy and TB preventive therapy will help save an additional 3 million lives over the next generation (Figure 1C). Thus, TB program budgets should be increased to enable reaching these high-risk groups. Yet ultimately, the fight against TB will not be won unless we also ensure that everyone, not just high-risk groups, has access to essential health services. No country will end the TB epidemic within its borders without ensuring that all have access to high-quality care, free from risk of catastrophic medical costs. Thus, achieving universal health coverage is essential to sustaining an end to TB.

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Investing in TB research and development

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EXECUTIVE SUMMARY: LANCET COMMISSION ON TB

While intensifying current efforts by rapidly scaling-up of proven strategies is necessary, ending the TB epidemic in high-burden countries will be greatly facilitated by new and better tools, and effective adoption of enabling technologies and programmatic innovation. In the near term, increasing the investment in diagnostic, therapeutic, and prevention R&D, as well as population, policy and implementation research to rapidly transform research findings into TB programs and policies, is likely to be ~~of~~ high impact~~yield~~.

Reaching these research goals will require tripling our global investment in TB R&D from $750 million per year to more than $2 billion per year during the next four years. However, this investment cost must be weighed against the cost of inaction; in India, for example, even with optimal implementation of all existing tools, unavoidable TB deaths will cost the economy at least US$32 billion each year over the coming 30 years. In Kenya, the cost of failing to introduce new tools will be as much as 10% of the country's GDP. While greater investment from high-income countries is imperative, high-burden middle-income countries, such as Brazil and South Africa, can transform the global TB R&D agenda through increased financial investment, collaborative networks, and incentivized interdisciplinary research partnerships.

Sustained financing

Everyone dedicated to achieving an end to TB—affected countries, donor nations, the private sector, foundations--must ~~significantly increase~~~~redouble~~redouble their efforts to finance current working strategies ~~that are working now~~ and, more importantly, ~~to~~ novel strategies that have the most potential to make a significant difference in the future. Crucially, high-burden countries can substantially increase financial resources to fight TB. By pooling finances, increasing the allocation of public resources to health, ~~pooling finances~~ and earmarking tobacco taxes to health, countries like Bangladesh, Zambia, China, and Indonesia can increase their annual TB expenditures more than five-fold over the next five years. The dividend of this investment is likely to be substantial: recent history demonstrates that those countries that have achieved extraordinary progress against TB reaped broader economic and health benefits that continue to this day.

Accountability and shared responsibility

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EXECUTIVE SUMMARY: LANCET COMMISSION ON TB

To ensure that the results required to end TB occur within a generation, clear accountability mechanisms are necessary. TB report cards for heads of states, national TB programs, donor agencies, and key TB stakeholders can help ensure that promises—~~-~~including financial promises—~~-~~are kept, and progress is made. They also can ~~also~~ ensure that corrective actions are taken, and that the financial, political, and programmatic barriers to delivering comprehensive TB treatment and care are removed. Accountability to ensure multisectoral action to address risk factors for TB, such as air pollution, tobacco control, and undernutrition, is also important.

We cannot create an enabling environment to rid a country of TB without engaging civil society and acknowledging it as a vital stakeholder in our efforts to end TB. Groups like the Treatment Action Campaign in South Africa and Volunteer Health Services in Ethiopia have become increasingly prominent voices for action against TB in their countries. We must strengthen ~~the~~ civil society's involvement in all aspects of TB programming~~,~~ and increase~~ing~~ their decision-making roles in planning, implementation, and accountability. Additionally, we must uphold and defend the rights of people with TB and those most at risk, and ensure we put in place policies and practices to protect them against stigmatization.

In conclusion, the question is no longer can TB mortality be reduced significantly~~to a level of no public significance~~. The question is whether we as a global community have the political will to take the necessary steps to end TB within a generation, including working towards the goal of achieving universal health coverage. We believe there are grounds for optimism. Ending TB is possible through rapid strengthening and expansion of our existing delivery systems with implementation of the evidence base we already know, matched with innovative science leading to new and better diagnostics and prevention and treatment approaches; and increased and sustainable financing. Above all, this Commission reiterates that there can be no room for complacency. We must act strategically and quickly to avert more premature deaths from this preventable and curable disease.

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EXECUTIVE SUMMARY: LANCET COMMISSION ON TB

PANEL A

Investment Priorities

The Commission recommends that the following ~~priority~~ investments be ~~made~~priorities to build a TB-free World. These investments are designed to fulfill the mandate of the Moscow Declaration and the UN High-Level Meeting on TB. In addition, they answer the question: *How should TB high-burden countries and their development partners target their future investments to ensure that ending TB is achievable?*

These Investment Priorities are predicated on the fact that UHC will eventually be adopted by each country as they work with development partners to create locally-owned and sustained solutions.

Priority 1. ~~Invest t~~To have the most immediate impact, ~~by~~ effectively and universally respond~~ing~~ to known TB cases in high-burden settings. ~~First~~Initially this entails providing high-quality treatment ~~of~~to known TB cases. ~~It is l~~Likewise, it is essential to detect, treat, and prevent TB in their family members and other close contacts~~, of people who are sick.~~ Where ~~drug-resistant~~DR- TB is prevalent, increase access to drug susceptibility testing and treatment for DR~~resistant forms of~~ TB. Achieving universal and high-quality patient- and family-centered care should, in most cases, be the top policy and budgetary priority and will require sustained efforts to improve the generally ~~poor~~ (could replace with limited or weak) performance of private sector providers.

Priority 2. Reach high-risk populations, by beginning with those most easily identified. Active case-finding and treatment ~~in~~of high-risk populations—such as people living with HIV, migrants, and prisoners—~~d~~demands adequate resources available to reach them~~se populations~~. At the same time, target these high-risk populations for TB preventive therapy, which is essential to achieve epidemiologic control. Only as high-risk populations are successfully covered should the general population be reached, primarily by the strengthening capacity of health system delivery and moving toward universal health coverage.

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Priority 3. Investments should be a shared responsibility, with development assistance for TB control increasing commensurate with the financial needs of individual low- and middle-income countries. Gradually redirect external assistance to middle-income countries for three purposes: ramping up control capacity in low-income countries; reducing the spread, particularly the cross-border spread, of DR -TB in all affected low- and middle-income countries; and financing the population, policy, and implementation research that will provide lessons learned and international sharing of best practices.

Priority 4. Increase investment to ~~to~~ accelerate R&D and bring new diagnostics, therapeutic strategies, and vaccines into play ~~in hand~~ to more quickly end the epidemic. Typically, budgets for new product development are outside of the purview of health ministries or development assistance agencies. Hence, strong advocacy to science ministries and research-oriented pharmaceutical companies is critical, including to ministries and companies in middle-income countries. Financing early uptake of new products will provide important signals to product developers.

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Priority 5. Hold countries and key stakeholders accountable for progress made towards ending TB. This entails establishing TB report cards to ensure that heads of states, national TB programs, and even regional and site-level clinics are held accountable for their responsibilities that contribute to ending the epidemic. These accountability mechanisms should not only assess progress, but also guarantee corrective actions are taken to remove obstacles to ending TB.

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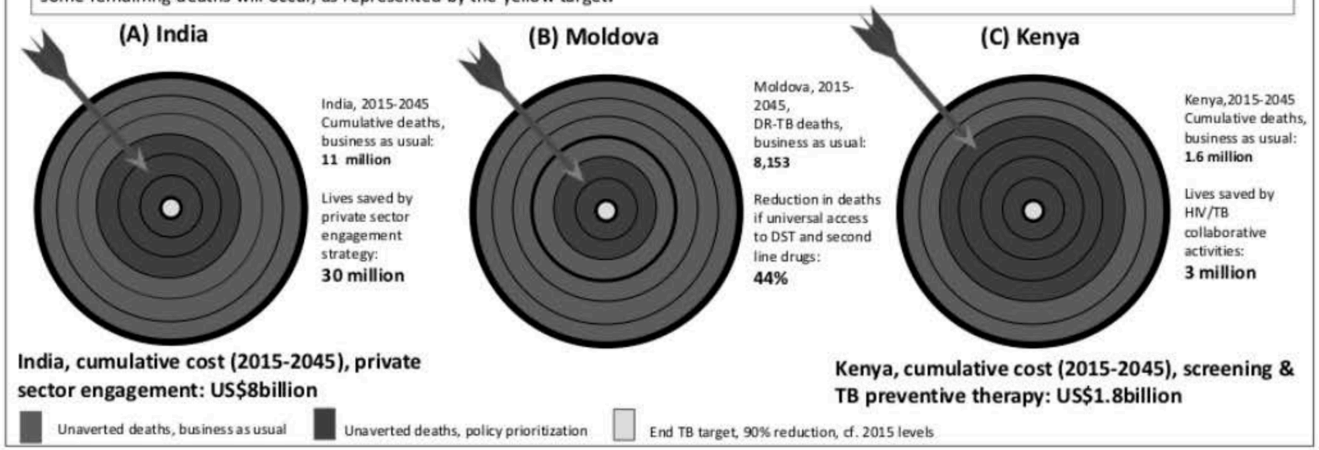
Ending TB means hitting the bullseye: Reducing TB deaths by 90% compared with 2015 levels. Here we present the number of deaths that will occur between now and 2045 if countries continue 'business as usual' (blue circle). Recognizing that reducing TB deaths will require different interventions in different countries, we illustrate how 3 specific policies in 3 different countries can reduce TB mortality significantly (red circle). For example, in India, strengthening private sector engagement will avert 30 million deaths (95% CI, 18-53 million) over the next generation. In Moldova, ensuring universal access to drug susceptibility testing and second-line drugs will reduce deaths from DR-TB by 44% (95% CI, 35, 51%). In Kenya, screening all people living with HIV for TB and offering TB preventive therapy to eligible individuals will avert four million deaths (95% CI, 2-4 million), reducing overall TB deaths by 70%. Ultimately, multiple interventions, as well as new tools and technology, are needed to hit the target and reduce TB deaths to 90% of the 2015 level (yellow inner circle). Figures (A) India, (B) Moldova and (C) Kenya; in each the projected cumulative deaths (2018-2045) in a 'business as usual' scenario is represented by the larger blue circle ; if countries successfully implement high-impact interventions, the projected number of deaths over this period will drop– this smaller red circle represents the remaining deaths still occurring even in spite of these interventions; even if countries hit the bullseye, some remaining deaths will occur, as represented by the yellow target.



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233 **Table 1: Assessing the impact of priority interventions in 3 different country-settings.**

Country/Policy	*Deaths Averted (Millions)*	*Deaths Averted (%)*	*Cost of Policy Implementation (Millions)*
India Private sector engagement	**30** 95% CRI (17.6-53.4)	**28** 95% CRI (16.7-46.3)	**$290m p.a.** 95% CRI (85-645)*
Kenya HIV/TB activities including TBT	**3** 95% CRI (1.9-4)	**20** 95% CRI (13.4-25.9)	**US$65 p.a.** 95% CRI 20- 113)**
Moldova Universal access to DST and Second line drugs	**0.04** 95% CRI (0.03-0.05)	**44** 95% CRI (34.7-51.4)	N/A

234
235 Results reflect cumulative incidence and deaths averted from 2018 to 2045, relative to a baseline scenario of extended current state. Private sector engagement, Optimised HIV
236 cascade and DST in new and retreated cases (with improved outcomes in second line treatment) were selected as interventions for India, Kenya and Moldova respectively.
237 *Does not include cost of second line drugs, ** does not include ART costs; TBT- TB preventive therapy
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Lancet Commission on TB

Section 0

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Introduction: Tuberculosis in the 21st Century

'Knowing is not enough; we must apply. Willing is not enough; we must do.'
Goethe

Progress against tuberculosis: moving forward, but not fast enough

In 1993, the World Health Organization (WHO) declared tuberculosis (TB) a public health emergency[1] and urged governments worldwide to significantly scale up their TB control efforts. A year later, WHO unveiled 'directly observed treatment, short course,' or DOTS, as its solution to the problem. In addition to using direct observation to improve adherence to standardized treatment, the DOTS package included microscopy-based case detection, political commitment, and better reporting of data. However, the DOTS strategy was neither comprehensive enough nor sufficient to reduce TB transmission. Latent TB, childhood TB, and drug-resistant TB (DR TB) received little attention in the original DOTS framework. The expanding HIV epidemic and the increasing scope of DR TB further undermined an approach reliant on ~~blunt~~ nonstandardized and imprecise diagnostic tools and passive case detection.

Despite gains made against the TB epidemic since then—and the subsequent introduction by WHO of an enhanced strategy to intensify TB control efforts[2,3]—the potential to dramatically reduce the rates of TB incidence and mortality worldwide as first proposed a quarter century ago have not been realized. In 2016, an estimated 10.4 million new cases of the disease occurred worldwide. Today TB remains a global public health emergency, responsible for more deaths than any other infectious disease; in 2016 ~~an~~ 1.74 million people with TB died from the disease, ~~; another~~including 400,000 people who died with HIV ~~were~~ coinfectioned ~~with TB at the time of their deaths~~.[4] Moreover, ~~it~~TB disproportionately affects the most marginalized and powerless members of society, including migrants, prisoners, children, the poor, and people living with HIV. In a cruel paradox, TB—first cured in 1949—continues to cause untold suffering, loss of life, and financial catastrophe for millions of people worldwide.

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The barriers to progress are complex and include economic, programmatic, and biological factors. TB festers because the disparate, economically disenfranchised populations most affected lack an insistent civil society voice to engage politicians and funders to advocate for transformative change. Public health programs targeting TB tend to be siloed, vertical endeavors, undercutting integrated, multisectoral strategies necessary to address underlying social determinants. The persistent but unappreciated effect of the stigma attached to this 'disease of poverty' has undermined both optimal care and financial investment. Because latent TB affects millions of people, TB cannot be eliminated without scaling up prevention and diagnosis efforts as well as treatment for those with active disease. For myriad reasons, however, preventive therapy has been difficult to scale up, even in high-risk groups that most need it. Finally, the perceived limited profit margins have been a disincentive to increased private investment for developing new diagnostics, therapeutics, and vaccines.

As a result of sluggish progress, more than 4 million TB cases still go undiagnosed or unreported each year, DR TB infections affecting hundreds of thousands of people continue to spread, especially in certain 'hot spots,' and annual TB-related deaths—especially in children—remain unacceptably high. The quality of TB care is suboptimal in high-burden settings, with long diagnostic delays[5,6] and high treatment dropout rates.[7] In addition, TB preventive therapy is not being offered to many people most at risk, despite compelling evidence that it saves lives.[8] Clearly, current TB care and prevention efforts are inadequate. Failure to scale up what we know works will cost billions of dollar over the next 30 years and lead to an enormous loss of life.[9] Unchecked, TB also threatens global health security , especially in countries with precarious economies. More can and must be done to halt the TB epidemic. To do so, moreover, is a social justice and human rights imperative—TB can be cured, and TB epidemics can be controlled, as successful programs in high-income settings have proved. Recognizing this, the global TB community outlined an ambitious End TB Strategy in 2014[10], [Panel], which has been incorporated into the UN Sustainable Development Goals (SDGs) to which all countries have committed.

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Global Leaders have made a strong political commitment to ending the TB epidemic

Despite the challenges, there is growing optimism that in many countries, ending TB within a generation is within reach—if the opportunity is seized and commitment to the goal maintained. Taken together, programmatic innovations, new health technologies, sustained global economic growth, increasing commitment to achieving UHC, and mounting political momentum to definitively address TB can all

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contribute to ending TB. A long-term political pledge, however, requires a clearly defined endpoint. Fortunately, examples exist of how substantive, sustainable progress against TB can be achieved: Japan,[11] China,[12,13] and Peru[14] have all demonstrated that rapid declines in TB incidence and mortality can occur with sufficient political will and financial investment. The High-Level Meeting of United Member States in September 2018 is a propitious moment to capitalize on political momentum, and to propose how ending TB within a generation can be achieved, and at what cost.

In this context, our commission set out to answer the question: ***How should TB high-burden countries and their development partners target their future investments to ensure that ending TB is achievable?***

Report Roadmap

In **Section 0,** we assess the persistent global burden of TB, review current TB control efforts, and appraise why greater progress has not been made. We highlight past, current, and future barriers to success, including the millions of TB cases not identified by existing public health programs; broken TB care cascades; social determinants that increase TB risk; biological factors such as HIV/AIDS and diabetes ~~mellitus~~; and the long-neglected inclusion of research and development as vital to accelerating progress. We frame the challenges of ending TB within a broader sustainable development agenda, and in the context of progress, or lack thereof, towards UHC.

Section 1 highlights proven strategies to reduce TB mortality in high-burden countries. In particular, we focus on strategies to respond comprehensively to known cases. This will involve addressing gaps in the care continuum through better use of data and principles of quality management. In addition we highlight the crucial importance of reaching high-risk populations, through active case-finding strategies, while also targeting these populations for TB prevention interventions. We emphasize the critical need for new models of private sector engagement to deliver high-quality care, and innovative ideas to optimize care for patients with DR TB.

The challenge TB now presents also has resulted from neglecting to identify TB research as a critical priority during the last quarter century.[15] While ending TB with existing tools is possible, new products can reduce cost, simplify implementation, or accelerate progress. In **Section 2,** we describe why current

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funding for TB research and development (R&D) must increase to expedite innovations in point-of-care diagnostics; safer, less toxic , shorter, ~~safer~~ and more effective treatment regimens; and a more effective TB vaccine. The economic rates of return on greater TB R&D investment are both substantial and invariably pro-poor.[16]

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Section 3 highlights how effective TB control represents one of the 'best buys' in global development, one that can produce substantial economic dividends for high-burden countries. We examine the potential to expand domestic TB financing through increased revenue generation and prioritizing health care, as well as from more innovative sources, including loans, gains in efficiency, and complementary non-TB resources. Efforts to end TB in the 21st century need to differ dramatically from those in the past. Rather than relying on a global campaign funded and led by foreign donors and focused on specific interventions, increasingly TB control efforts will require domestic resources and full country ownership.[17] We discuss how foreign donor support can still play a critical role in 'transitioning' countries to full country ownership by protecting the TB risk pool and strengthening strategies that ensure sustainable domestic funding for TB control efforts.

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Section 4 calls for a new era of accountability and a reinvigorated cadre of political leaders committed to doing their part to accelerate the elimination of TB worldwide. Heads of states, national TB programs, and even regional and site-level clinics must be held accountable for their performance in contributing to ending the epidemic. We advocate for an independent review mechanism to evaluate the performance of all major global stakeholders engaged in TB programming.

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Section 0. TB – a major public health threat

In September 2018, 193 heads of state representing nearly every TB-affected country endorsed the UN SDG of ending the TB epidemic by 2030, pledging to stop TB within their respective country borders within a generation. This section presents a snapshot of TB today and describes current and future barriers to TB control efforts that must be addressed to ensure success.

TB-related mortality and the persistent burden of TB infection and disease

TB-related mortality: Globally, the TB mortality rate has declined approximately 3 percent per year since 2000, or 37 percent overall between 2000 and 20156. While this decline reflects the impact of DOTS, it also occurred commensurate with a decrease in poverty-related drivers of TB and with economic growth. As evidence of this, Cambodia, Ethiopia, Myanmar, Pakistan, and Uganda all achieved annual average rates of decline in TB mortality of more than 6%, and China of 7.7% (Table 0.1). Declining mortality rates were inversely associated with a decreasing sociodemographic index in the Global Burden of Disease (GBD) report.[18]

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This progress aside, TB mortality, especially in people living with HIV and in children is substantial.[4,19] Three-quarters of all TB deaths occur within just eight countries (Figures 0.1 and 0.2). Furthermore, rates of TB mortality have declined much more slowly than for most other infectious diseases (Table 0.2), and the declines are far lower in low- and lower-middle income countries compared with elsewhere (Table 0.3). In many parts of sub-Saharan Africa and Southeast Asia, TB remains a leading cause of years-of-life lost. Moreover, TB ranks as the 9th leading cause of death and the 12th leading cause of years-of-life lost worldwide.[20]

TB incidence: An estimated 10.4 million people (90 percent adults, 65 percent male) became ill with TB in 2016. Seven countries in Southeast Asia and Africa (India, Indonesia, China, Philippines, Pakistan, South Africa, and Nigeria) accounted for two-thirds of all new cases worldwide. Overall, TB incidence has fallen at a rate of approximately 1.4% per year since 2000 and 1.9% per year since 2015 – far less than the rate needed to achieve WHO End TB targets[4] (which propose that incidence rates decline annually from 4-5% by 2020 and to 10% by 2025 to achieve the milestone reductions in cases), and less than declining trends in mortality. Notably, high-income countries had the greatest reductions in TB incidence between 2000 and 2016, although they also exceeded 4% per year in Zambia (4.4%) and Ethiopia (5.4%)

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(Table 0.4). In contrast to mortality trends, the GBD annualized rates of change in TB incidence (2000-2015) showed an increased incidence in countries in the lowest sociodemographic quintile.[18] These discrepancies between trends in mortality and incidence highlight how few TB high-burden countries have implemented innovative case-detection approaches (Figure 0.3).[21,22]

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TB Prevalence: Between 2000 and 2016, 32 national TB prevalence surveys were performed in 26 countries.[4] These studies consistently found a higher prevalence of TB than previously estimated based on less precise information such as case notifications. The resulting revised incidence estimates highlighted large numbers of undiagnosed or unreported TB cases in many countries. Prevalence surveys also revealed that people with TB often sought care for TB symptoms that health care workers failed to identify. Other individuals did not recognize the seriousness of their symptoms and had not sought care. All prevalence surveys in the last decade have demonstrated a higher burden of TB among men, with male:female ratios ranging from 1.2 (in Ethiopia) to 4.6 (in Viet Nam).[4] The higher disease burden in men, combined with larger detection and reporting gaps, highlight gender differences in accessing care, that maybe related to financial barriers and stigma,[23] but also suggest that male-friendly strategies to improve access to and use of health services are required.[24] In addition, prevalence surveys between 2009 and 2015 highlight a trend towards an increasing TB prevalence in older individuals (>55 years) in Asia and some African countries. However, in Ethiopia, Nigeria, Zambia, Uganda, Gambia, and Sudan, prevalence peaks among those aged 35-54 years, consistent with impact of HIV co-infection in these countries.

Why haven't we made more progress?

The reasons for our slow progress are complex. Health system frailities; lack of investment in control efforts, and in research towards developing new medical tools; reliance on simplified, one-size-fits-all approaches; and biological factors such as HIV ~~/AIDS~~ co-infection and the spread of drug resistance are all to blame. TB ~~is~~ also is 'a disease of the shadows,' disproportionately affecting those communities with the least powerful constituencies to effect change.

Deaths from TB fell rapidly in western Europe and the United States commensurate with improvements in living standards and access to potent chemotherapy. The decline in TB cases in high-income countries and the lack of a ~~loud~~ powerful civil society voice in high-burden countries have undermined efforts to garner the same political support or domestic financial investment as for other diseases. Failure to appreciate the profound negative economic impact of the epidemic and advocate for increased donor

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financing in high-burden, low-income countries has hampered efforts. In many of the highest burden countries, chronic under-funding and lack of policital will have had a profound disabling impact on TB programming, but also explain why, ~~forty~~40 years after the Alma Ata Declaration, half the world's population still lacks access to comprehensive health care services. In addition, R&D has been neglected despite being a priority pillar of the WHO End TB strategy.[10] In 2018, most low- and middle-income countries are still using tools (e.g., smear microscopy and the BCG vaccine) developed nearly a century ago, and access to new tools remains suboptimal.[25]

Broken care cascades. missing millions and poor quality of care

Turning the tide on TB requires early, accurate case detection together with rapid initiation of and adherence to effective treatment that prevents transmission. To achieve this, national TB programs in high-burden regions must scale up active case-finding strategies, targeting high-risk populations, rather than relying on passive case finding alone. As prevalence surveys highlight, many individauls with TB are asymptomatic. Waiting for patients to become sick enough to seek care has not markedly reduced rates of TB transmission and incidence .[26-28] A more aggressive approach is needed. Unfortunately, active case-finding strategies, even in the highest risk populations, are not widely implemented because of cost concerns and the dearth of population-level data to inform best practices. In addition to finding those individuals suffering from TB who are not yet presenting, more needs to be done to address the health system barriers that make~~ing~~ accessing care so challenging. Evidence from high-burden countries increasingly demonstrates that many people with TB either cannot access healthcare, or they visit facilities that cannot diagnose and treat TB.[29] Across five countries–Kenya, Ethiopia, Indonesia, the Philippines, and Pakistan–the first health care contact for more than three-quarters of TB patients were facilities that did not have access to TB diagnostic capability. Furthermore, 66% of patients first sought care in private or informal facilities, which had considerably less diagnostic capacity than public facilities. Even in countries that had scaled up access to Xpert MTB/RIF (Xpert; Cepheid, Sunnyvale, CA, USA), a rapid and sensitive molecular TB diagnostic, patients were not presenting at venues with this resource. Consequently, less than 50% of all estimated TB cases achieve the first step in care required for a durable cure. These results confirm the findings of numerous other studies demonstrating the many programmatic, geographic, and financial barriers[30,31] that prevent people with TB from accessing healthcare.[5]

Unfortunately, not only is *access* highly variable, so too is the quality of TB care in many high-burden countries. The Lancet Global Health Commission on High-Quality Health Systems (HQSS) defined high-

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quality health systems as those that optimize health in a given context by consistently delivering care that improves or maintains health, being valued and trusted by all people, and responding to changing population needs (Kruk M et al, Lancet Global Health, 2018). This Commission concluded that poor quality is common across conditions (including TB, which was highlighted as an important example) and countries, with the most vulnerable faring the worst. As figure 0.x demonstrates, even the foundations of TB programs are weak, resulting from chronic under-funding, limited access to new tools, and inadequate implementation of policies.

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Recent studies have highlighted substantial gaps in the TB care continuum for all forms of TB cases: active, DR, latent, and those in children (Figure 0.4). In India, an analysis of the public TB care cascade found that only 60% of the country's 2.7 million incident TB cases were diagnosed, and only 452% of those completed treatment.[32] For patients with MDR-TB, only 14% completed treatment, and 11% remained disease-free at one year. A similar study in South Africa found that only 82% of 532,005 TB cases were diagnosed, and less than 54% of drug-susceptible TB cases completed treatment.[33] Of those with rifampicin-resistant TB, only 22% completed treatment (cite Figure 0.4) . Simulated patient studies in 3three countries show that most primary care providers are unable to diagnose TB, and referral linkages to the NTP system are weak. In India, China, and Kenya, only 28% to 45% of mystery patients were correctly managed by primary care providers.[34-36]

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Simply put, the current global capacity to diagnose, link to care, treat, and cure TB patients does not match the massive burden of disease that exists. The public health implications, as well as the poor clinical and financial implications[37] for patients, are self-evident. Substantially reducing TB mortality and incidence will require significantly increasing both the coverage and the quality of TB services across the entire care continuum.

Achievement of Global Targets Require Engagement of Private Providers

Of the 4 million "missing" TB patients in 2016, 63% of them are in six countries where primary care is dominated by private providers and > more than 65% of initial care-seeking is in the private sector (Table 0.x [previously 1.3.1). However, in these countries, private provider notifications are just 18% of total TB notifications and 9% of estimated TB incidence. Based on data from TB prevalence surveys and private sector drug sales,[38] a considerable proportion of TB patients are treated in the private sector but not reported. Given the dominance of private health care in countries with the largest share of the "missing" TB patients, national and indeed global TB goals will not be met unless private providers are

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engaged on a scale commensurate with their role in primary care. Modeling studies also suggest that untreated or poorly-treated patients in the private sector are a major source of transmission.[39] This is due to delay in diagnosis and treatment initiation among private patients, as well as recurrent TB among private patients who were inadequately treated. Therefore, improving the diagnosis and treatment of patients seeking care in private facilities is an opportunity to rapidly reduce TB transmission. Engaging private providers can also reduce unnecessary morbidity and mortality caused by inappropriate treatment, drug resistance caused by undetected MDR TB and incomplete treatment, and catastrophic expenditures and impoverishment.

Hot spots and high-risk populations demand targeted solutions

Global and regional data camouflage localities where the TB epidemic continues to grow unabated; many different micro-epidemics exist, and the risk of both acquiring and dying of TB are unevenly distributed across society. Even adjacent neighborhoods may have markedly different TB prevalences, as recent analysis from Chennai, India, illustrates.[41] Such regional variations reflect social and environmental determinants, which include living in densely populated areas[42-44] and working in occupations, such as health care or mining, that increase the risk for TB.[45-47]

Within most countries, TB incidence and TB-related mortality vary geographically, and they affect specific populations more than others. HIV co-infection is the leading risk factor for developing and for dying from TB:[48] Ten percent of all new active TB disease cases and 25% of all TB-related deaths occur in HIV-infected individuals. However, co-infection with TB and HIV varies widely according to geography. In parts of southern Africa, more than half of all TB patients are HIV-infected; in China and India, only a small fraction of TB cases are co-infected with HIV.[49]

Host factors, such as low body mass index,[50] tobacco use, silicosis and biomass fuel exposure,[51,52] diabetes, [53] and heavy alcohol use[54,55] also can influence disease progression in individuals exposed to TB. As Table 0.5 demonstrates, cigarette smoking and air pollution contribute substantially to the global TB risk. Accurately estimating how many people have each of these risk factors is not possible. However, based on the number of active smokers worldwide and the rapidly expanding number of people with diabetes, we can infer that the proportion of TB cases due to diabetes is five times higher and the proportion due to smoking is eight times greater than the proportion due to HIV/AIDS.[56-58]

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While TB incidence tends to be higher in specific geographic hotspots, such as densely populated slums, and among certain high-risk populations, i.e., HIV-infected individuals, the vast majority of people acquiring TB each year are not 'high risk' and do not acquire TB from 'hotspots.' Airborne transmission of the infection and clinical latency mean that TB disease can affect anyone, at anytime.

The importance of prevention

Prevention strategies have been a central tenet of efforts to eliminate all major infectious diseases. The eradication of smallpox, for example, followed ~~included~~ a multi-pronged approach that included finding cases and offering vaccination to contacts of infected people residing in epidemic hotspots.[59] Mass preventive therapy has been used to combat both *Chlamydia trachomatis* and *Onchocera volvulus*.[60] Similarly, ending TB as a disease of public health significance must entail a comprehensive, cogent prevention agenda. Because the human reservoir of *M. tuberculosis* infection is enormous,[61] overwhelmingly asymptomatic, and long-lived, identifying individuals who are at highest risk of progression to disease, who would thus benefit the most from preventive therapy, is crucial..

The benefits of preventive TB therapy have been known for more than 60 years. Pioneering studies in the 1950s–1960s provided overwhelming evidence of the efficacy of isoniazid in preventing active TB in children,[62] Alaskan Native populations, residents of congregate living facilities such as mental hospitals, and household contacts of TB patients. [63] Subsequent work has further documented benefits of preventive therapy for individuals with evidence of recent infection, those with radiographic evidence of prior untreated TB, [64]people with HIV infection,[65] recipients of immunosuppressive therapy such as TNF-alpha inhibitors,[66] and other immunocompromised individuals.[67]

Large population-based studies of TB preventive therapy and mathematical models both suggest that preventive treatment of TB infection, as part of a comprehensive approach that includes active case-finding and prompt, effective treatment, can sufficiently reduce population-level transmission to interrupt the cycle of infection, illess, and death.[68,69] Unfortunately, despite abundant evidence of its efficacy, the use of preventive therapy globally has been limited,[70] as TB control programs in LMICs have focused almost exclusively on detection and treatment of individuals with active TB disease.

Moreover, the development of new TB vaccines is essential to TB prevention and elimination. The Bacillus-Calmette-Guérin (BCG) vaccine currently used to protect infants and young children from the most severe consequences of TB disease is more than 100 years old.

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The problem of drug-resistant TB

Resistant forms of TB account for about 1% of cases in many countries in sub-Saharan Africa, western Europe, and North America, while DR TB prevalence is more than 20% in several countries from the former Soviet Union, such as Azerbaijan, Belarus, Kyrgyzstan, and Moldova.[71] Within individual countries, MDR TB prevalence can vary by a factor of ten at the sub-district level and even more from one health center to the next.[72,73] The largest number of DR TB cases are in India (which has witnessed the emergence of so-called 'totally drug-resistant' strains)[74] and China (where one-quarter of all active TB disease cases are resistant to either isoniazid or rifampin).[75] Increasing data demonstrate that most DR TB cases reflect transmission rather than initial acquisition.[76-78] Thus, a high priority for curbing DR TB is to identify and target 'hotspots' of DR TB transmission.[79] In parallel, efforts are urgently needed to develop new, safer, less toxic, shorter, and more effective drug regimens for TB.

Addressing social determinants

Fundamentally, TB is a disease of poverty.[80-83] Most often it causes substantial losses in productivity for already poor individuals (3-4 months of work), families (30% of yearly household earnings), and countries (4-7% of GDP).[84] Social determinants that contribute to TB risk are linked both directly and indirectly to social and economic vulnerabilities.[81] Surveys in seven countries demonstrate that patients who develop TB often face catastrophic costs (>20% of household income) just to access care to diagnose and treat their TB.[30,31,85-88] In Viet Nam, for example, 63% of TB-affected households experienced catastrophic costs, 38% took out loans or sold assets (so-called "dissavings"), and 27% reported serious financial burdens related to TB-related costs.[89] Significant social and economic burdens make TB patients less likely to present for care, complete TB testing, and initiate and adhere to treatment,[82,90] leading to increased transmission, morbidity, and mortality.[21,51,91-95] The financial impacts of TB disease are profound and long lasting; as we highlight in Panel 6, individuals suffering from TB in rural India experienced profound financial hardship even seven years after completing TB treatment.

Emerging trends and evolving threats

As history demonstrates, the global TB epidemic is not homogenous, characterized by a gradual decline in incidence, but rather a heterogeneous collection of micro-epidemics in which transmission in each setting is driven by different catalysts,[96] from HIV-induced immune defects to inadequate diagnosis and treatment.[97] In settings where increased attention and resources have been devoted to controlling TB (for example, New York City,[98] Alaska,[99] and China[75]), remarkable successes have been achieved. But in regions where facilitators of transmission have been left unaddressed (~~HIV in sub-Saharan Africa~~ and incarceration in eastern Europe, for example), TB has resurged. To prevent the 'worst of history'

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repeating itself, TB control programs must anticipate and respond to dynamic demographic, environmental, and socio-economic trends, mapping the epidemic in each individual setting to clearly understand how the epidemic is evolving.

Emerging trends that threaten global TB control efforts include the following:

- *Aging population*: With longer average life expectancies and declining fertility rates, by 2050 there will be 2 billion people over the age of 60 years. Acknowledging higher TB incidence and mortality in vulnerable elderly in both low-burden[100-102] and high-burden countries,[13,103] TB control programs will need to employ targeted strategies to address this demographic shift.[104]
- *Increasing prevalence of non-communicable diseases (NCDs):* The prevalence of diabetes and other non-communicable diseases is increasing dramatically. Countries in Asia, particularly India and China, will bear the brunt of the diabetes epidemic over the next decade.[105] Diabetes, chronic lung disease, and other important NCD drivers of TB epidemics are highly prevalent in many high-burden countries.
- *Climate change:* Climate change and environmental biodegradation may undermine TB control efforts in many low-income countries through increased food insecurity, mass migration, and social dislocation, especially in settings where air pollution and undernutrition already pose a substantial risk for TB.
- *Urbanization:* The high incidence of TB in the favelas of Rio de Janeiro provides evidence of how TB transmission concentrates in poor, urban communities.[79,106] Given estimates that 66% of the global population will live in cities by 2050, including 2 billion in slums, the potential threat of increased urbanization to TB control cannot be overstated.

The way forward – Creating a path for success

Currently, we are not on track to meet the UN SDG and WHO End TB goal of eliminating TB by 2035;[3,107]many high-burden countries will not achieve the 2030 End TB targets for at least another hundred years. Nonetheless, substantial progress can be made towards these goals, based on the example of several countries—including high-burden countries in Africa and Asia—where TB mortality rates have declined dramatically during the past 15 years. These countries have demonstrated trends in mortality rate decline, that if applied to many other countries could help end TB mortality as a serious public health threat within a generation. If this progress can be replicated elsewhere, many countries

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can end TB within a generation, (Figure 0.5). To replicate these rates of decline will require substantial investment of resources, targetted to a few key priorities. We discuss these priorities further in Section 1.

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681 **Appendix**

Table 0.1. Tuberculosis mortality in 2000 and 2015: 30 countries with the most TB deaths in 2015[a]

	Country	Deaths, 2000 (thousands)	Deaths, 2015 (thousands)[a]	Cumulative % deaths, 2015 (as % of total TB deaths)	TB deaths as % of total deaths, 2015	Death rate, 2000[b]	Death rate, 2015[b]	Rate of decline in deaths, 2000-2015[c]	Rate of decline in death rate, 2000-2015[c]	Demographic headwinds
	World	1700	1400	100%	2.40%	27	19	1.30%	2.50%	1.20%
1	India	580	470	34%	4.90%	55	36	1.40%	2.80%	1.50%
2	Nigeria	110	180	47%	7.90%	90	96	-3.00%	-0.40%	2.60%
3	Indonesia	110	98	54%	5.30%	52	38	0.70%	2.00%	1.30%
4	Bangladesh	82	69	59%	8.00%	62	43	1.10%	2.50%	1.40%
5	DR Congo	30	48	63%	6.60%	62	62	-3.20%	-0.10%	3.20%
6	Pakistan	88	44	66%	3.20%	63	23	4.60%	6.70%	2.10%
7	China	99	34	68%	0.30%	34	2	7.20%	7.70%	0.50%
8	Philippines	35	30	71%	4.50%	45	30	1.00%	2.70%	1.70%
9	Tanzania	24	29	73%	7.00%	70	54	-1.30%	1.70%	3.00%
10	Myanmar	60	26	75%	6.00%	126	48	5.60%	6.40%	0.80%
11	South Africa	24	25	76%	4.70%	53	46	-0.30%	0.90%	1.30%
12	Ethiopia	57	24	78%	3.40%	86	24	5.70%	8.40%	2.70%
13	Mozambique	17	19	80%	6.60%	91	67	-0.90%	2.00%	2.80%

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14	Russian Federation	33	18	81%	0.90%	22	13	3.90%	3.80%	-0.10%
15	Viet Nam	23	15	82%	2.80%	29	16	2.80%	3.80%	1.00%
16	DPR Korea	4	15	83%	6.50%	18	60	-8.80%	-8.20%	0.60%
17	Afghanistan	14	13	84%	5.10%	69	41	0.10%	3.50%	3.30%
18	Angola	10	11	85%	3.20%	65	44	-0.80%	2.60%	3.40%
19	Madagascar	11	10	86%	6.30%	68	42	0.40%	3.30%	2.90%
20	Ghana	10	10	86%	4.30%	52	36	0.00%	2.50%	2.50%
21	Kenya	5	9	87%	2.80%	15	19	-4.20%	-1.60%	2.60%
22	Thailand	13	8	88%	1.50%	20	12	2.70%	3.30%	0.50%
23	Cambodia	16	8	88%	8.50%	128	51	4.40%	6.10%	1.60%
24	Sudan	8	8	89%	2.60%	29	20	0.10%	2.50%	2.40%
25	Cameroon	11	7	89%	2.80%	68	29	3.10%	5.60%	2.50%
26	Ukraine	12	7	90%	1.00%	24	15	3.70%	3.10%	-0.60%
27	Somalia	6	7	90%	4.80%	81	61	-0.60%	1.90%	2.50%
28	Brazil	9	6	91%	0.50%	5.2	3	3.00%	4.10%	1.10%
29	Uganda	10	5	91%	1.80%	42	14	4.20%	7.50%	3.30%
30	Nepal	4	5	91%	2.80%	17	18	-1.70%	-0.50%	1.20%

Source: World Health Organization (2017), Mathers et al (2018)

Note: [a]Per standard classification in the WHO's Global Health Estimate, and in the IHME Global Burden of Disease, TB deaths in HIV-infected individuals are classified as AIDS deaths and therefore are not included here. [b] The death rate is expressed as TB deaths per 100,000 population per year. [c] This is the average annual rate of decline 2000 - 2015, expressed as percent per year. A negative rate of decline indicates rate of increase.

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Note: The following countries have achieved average rates of decline in death rates of 6% or more annually from 2000 - 2015: Cambodia, China, Ethiopia, Myanmar, Pakistan, Uganda. The following countries have experienced an increase in the TB death rate from 2000 - 2015: Democratic Republic of Congo, Democratic People's Republic of Korea, Kenya, Nepal, Nigeria.

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Table 0.2. Major infectious causes of death, levels in 2015 and rate of change 2000 - 2015

	Total deaths (thousands)	Deaths by age group in 2015 (% of total)			Rate of decline, total deaths, 2000 - 2015 [a]
		0 - 4 years	5 - 69 years	70+ years	
Tuberculosis [b]	1400	5%	70%	25%	1%
HIV/AIDS [b]	1100	8%	91%	1%	2%
Diarrheal disease	1400	38%	36%	26%	3%
Vaccine preventable disease	270	76%	22%	2%	9%
Meningitis and Encephalitis	400	29%	62%	9%	2%
Malaria	440	71%	25%	4%	4%
Respiratory infections	3900	1%	30%	69%	0%

Source: World Health Organization (2017), Mathers et al (2018).

Note: [a]This is the average annual rate of decline 2000 - 2015, expressed as percent per year. A negative rate of decline indicates rate of increase. [b]The HIV/AIDS death totals include deaths of the individuals co-infected with tuberculosis.

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Table 0.3. Tuberculosis deaths and death rates by country income group, levels in 2015 and rates of change 2000 - 2015

Country income group [a]	Population, 2000 (millions)	TB mortality, 2000		Population, 2015 (millions)	TB mortality, 2015		Rate of decline 2000 - 2015 [c]	
		Deaths (thousands)[b]	Death rate (per 100,000 population)		Deaths (thousands)[b]	Death rate (per 100,000 population)	Deaths	Death rate
World	6100	1700	27	7300	1400	19	1.30%	2.50%
Low-income	640	380	60	900	330	36	1.00%	3.30%
Lower-middle income	2100	1000	50	2700	910	34	0.90%	2.50%
Upper-middle income	2200	190	8.8	2500	110	4.4	3.70%	4.60%
High-income	1200	52	4.3	1300	30	2.4	3.40%	4.00%

Source: World Health Organization (2017), Mathers et al (2018).

Note: [a] Country income groups are defined in the World Bank World Development Indicators for the year (XX) (World Bank, XX).[b] Per standard classification in the WHO's Global Health Estimate, and in the IHME Global Burden of disease, TB deaths in HIV-infected individuals are classified as AIDS deaths and therefore not included here.[c] This is the average annual rate of decline 2000 - 2015, expressed as percent per year. A negative rate of decline indicates rate of increase.

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Table 0.5. Global Prevalence, Relative Risk and Attributable Fraction for Incident Tuberculosis

Risk Factor	Global Prevalence	Relative Risk	Attributable Fraction	Reference
HIV/AIDS	0.008	8.3	5.5	World Health Organization (2009)
Undernourishment	0.11	2.1	10.7	Lonnroth (2010)
Diabetes Mellitus	0.085	3	14.5	Jeon and Murray (2008)
Heavy alcohol use	0.075	2.9	12.5	Lonnroth (2008)
Cigarette Smoking	0.21	2.6	25.1	Lin, Ezzati, Murray, and Slama (2007)
Indoor air pollution	0.41	1.5	17	Lin, Ezzati, and Murray (2007)

Source: Bloom et al (2017) (by permission).

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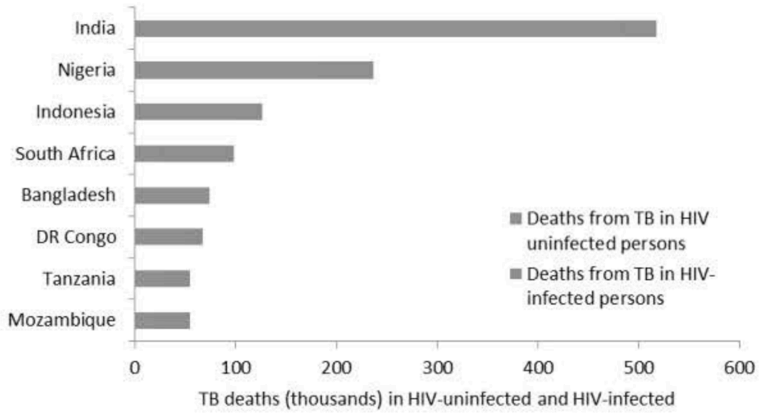
Figure 0.1. TB mortality among HIV-uninfected and HIV-infected individuals (thousands) in the 8 countries with the highest total TB mortality



Source: WHO (2016)

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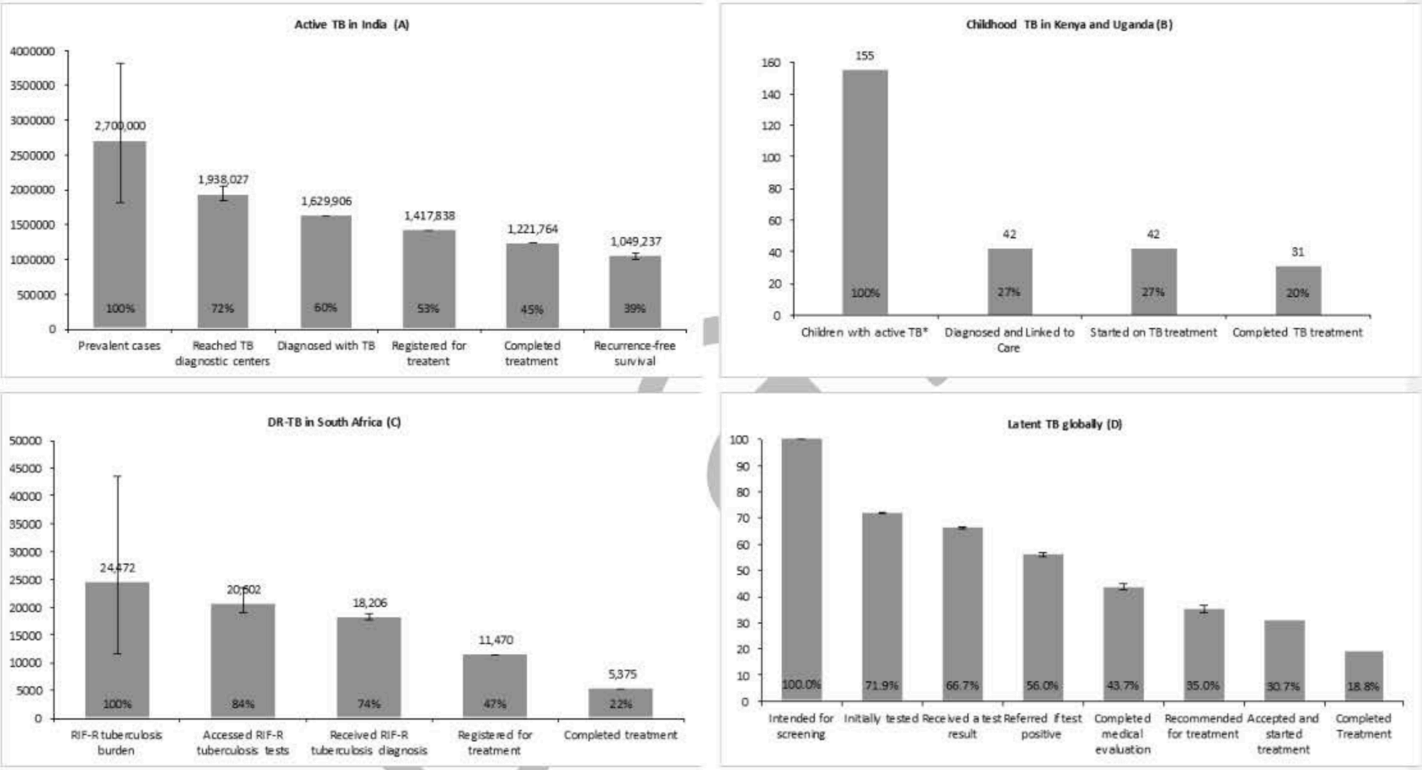
692 **Figure TB cascades of care**



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694 Source: (A) Subbaraman et al (2016) (B) Mwangwa (2017) (C) Naidoo et al (2017) (D) Alsdurf H et al, 2016

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699 **Figure 0.x. Quality of TB care, mapped to the Lancet Global Health Commission on High Quality Health Systems (HQSS) in the SDG Era**

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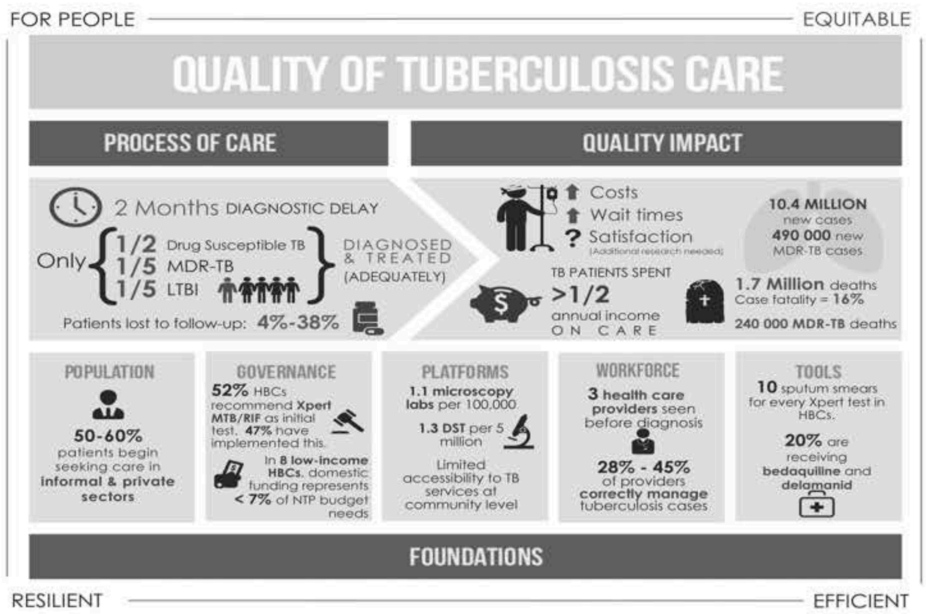

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Figure 0.5.1 When each country will reach End TB mortality targets, based on each country's current trend in decline in TB death rate*

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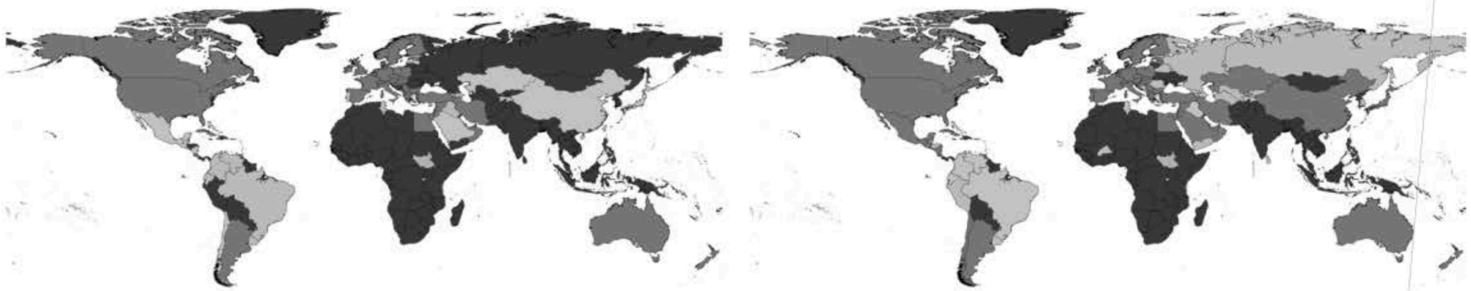
Map A: Achieving End TB target in 2015

Map B: Achieving End TB target in 2030



Already at goal (TB mortality <2/100,000 or TB mortality <90% of 2015 level in 2030 or <95% of 2015 level in 2035

Achieving End TB target within 15 years

Achieving End TB target within 30 years

Unable to achieve End TB target within timeframe

* Average mortality rate decline calculated as the average rate of decline in mortality rate in each country between 2000 and 2015.

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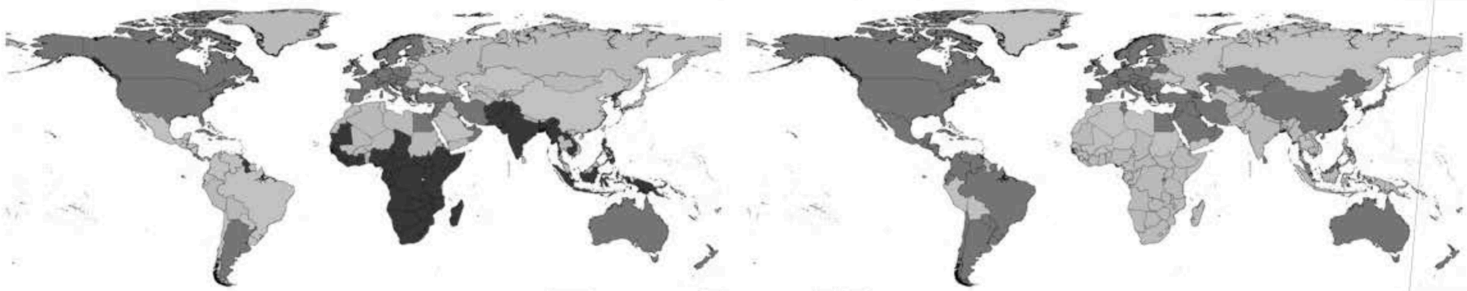
Figure 0.5.2 Progress towards ending TB: reaching End TB targets based on accelerated rate of decline in TB death rate**

Map C: Achieving End TB target in 2015

Map D: Achieving End TB target in 2030



Already at goal (TB mortality <2/100,000 or TB mortality <90% of 2015 level in 2030 or <95% of 2015 level in 2035

Achieving End TB target within 15 years

Achieving End TB target within 30 years

Unable to achieve End TB target within timeframe

** Average annual rate of decline in mortality rate of 7.7% applied to all countries (This estimated decline in mortality has been achieved by highest performing countries, 2000-2015).

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Lancet Commission on Tuberculosis

Section 1

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LANCET COMMISSION ON TUBERCULOSIS: SECTION 1

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Section 1: Introduction & Recommendations

Several high-performing countries have demonstrated that substantive declines in TB mortality can be achieved by using existing tools to scale~~ing~~ up evidence-based, best-practice interventions.~~, using existing tools.~~ A comprehensive approach that prioritizes responding to known TB cases through delivery of patient- and family-centered programs, while also reaching high-risk populations with screening and preventive services, can achieve results. This requires focusing resources to ensure high-quality services are available to diagnose and treat all forms of TB in both the public and private sectors. It also involves investing in strategies to find those suffering from TB in high-risk communities and scaling up preventive interventions in these communities. Although no one approach fits all countries, we highlight policy priorities that can inform domestic budget allocations and donor investments in high-burden countries (section 1.1-1.3), and also discuss the specific challenges faced by high-burden countries where private sector care is important (section 1.4) and where DR-TB is prevalent (section 1.5~~6~~). To complement these recommendations, we present modeling analysis from three countries with different epidemiologic profiles – Kenya, India, and Moldova.

The most important policy priorities summarized in this section include the following:

- Invest to have the most immediate impact by effectively and universally responding to known TB cases in high-burden settings. First this entails high-quality treatment of known cases. It is likewise essential to detect, treat, and prevent TB in family members and other close contacts of people who are sick.
- Reach high-risk populations, beginning with those most easily identified. Active case-finding and treatment in high-risk populations demands adequate resources available to reach these populations. At the same time, target these high-risk populations for TB preventive therapy, which is essential to achieve epidemiologic control. Only as high-risk populations are successfully covered should the general population be reached, primarily by the strengthening capacity of health system delivery and moving toward universal health coverage.
- Prioritize private provider engagement through strategies that improve quality, build partnerships, and encourage accountability, especially in high-burden countries with dominant private sector care.

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- Provide universal access to drug susceptibility testing (DST, as a minimum to rifampicin) for all individuals diagnosed with TB, especially in DR-TB high-burden settings, and ensure access to second-line DST for all individuals diagnosed with RR-TB

Section 1.1 Ensuring delivery of high quality, person-centered services

To respond effectively to people suffering with TB and to reduce delays in their diagnosis, treatment, and cure, TB services must reach patients where they seek care. A thorough assessment of TB epidemiology and care-seeking behavior is necessary to determine where resources should prioritized and what gaps in 'delivery gaps'[1] should be addressed first [Figure 1.1]. In all contexts, the first priority must be ensuring universal accesss to high-quality, person-centered TB care for individuals seeking care but not yet diagnosed, and those diagnosed, but not cured.

Unfortuantely, delivery of person-centered TB services to those who are seeking care is difficult in many high-burden settings for several reasons: first~~ly~~, individuals with TB are often not being identified and appropriately diagnosed in a timely manner;[2,3] second~~ly~~, once diagnosed, they are not started on or supported to complete treatment to ensure a durable cure. Other factors such as financial barriers and stigma, that undermine access to care for people suffering with TB are discussed elsewhere in this report. Here we assert the importance of patient pathway analyses (section 1.1.2), and network optimization tools (section 1.1.3) to ~~optimize~~ best align~~ment of~~ TB services with patient care-seeking behavior, and close these TB delivery gaps. In addition, we highlight the role of quality management solutions to improve the quality of TB services provided (section 1.1.4). These strategies can ~~help ensure~~ lead to higher TB cure rates and improve the efficiency of care delivery to ensure equity and control costs. They are applicable to both NTPs and non-state sector (private providers, nongovernmental organizations, etc) in expanding TB service coverage and quality. While these strategies are insufficient alone to end the epidemic, by redressing inequities in access, improving efficiencies in delivery, and protecting patients from physical and financial hardships, these interventions are integral to robust health systems and the broader SDG agenda.[4]

1.1.1 Patient-centered care requires data consolidation to set priorities

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As noted in section 0, recent studies have demonstrated that many patients, especially those in high-burden countries, cannot access services that diagnose or treat TB.[5,6] In Ethiopia, for example, only 46% of patients presenting to care with TB symptoms are likely to visit a facility that has TB diagnosis services[7]; in Indonesia, the estimate is 24% of patients.[8] Optimal use of available data in such settings can enable patient-centered approaches to TB care. For example, triangulation of local epidemiologic data, health system utilization data, and data on the TB care-seeking behavior of people and communiites with TB can help identify where diagnostic resources or treatment capability need to be reallocated for maximal impact and efficiency.[9]

Figure 1.1.2 illustrates how populating a matrix with routine surveillance and survey data, and with evidence related to patient interactions with their health and social systems, can be used to set patient-centered outcomes. The matrix can then be used to help estimate the relative magnitudes of the challenges facing patients and drive efforts toward the desired outcomes.

1.1.2 Patient pathway analyses to ensure all patients have access to services

Currently, global and national planning processes for TB prevention and care services continue to focus on "where/how can we provide services to align with our service capacity" rather than responding to local evidence that would inform "where/how should we position services to best reach and serve patients?" Despite increased efforts to find missing TB cases over the past three to five years, the proportion of estimated TB patients who are notified has plateaued. A paradigm shift is needed. Understanding where patients access health services and where they get 'lost' before achieving a cure is the first step to prioritizing efforts and planning the placement of services to meet patient needs and preferences.

Patient pathway analyses (PPAs) can enable programs to better understand how well patient care-seeking and TB service availability align. They highlight system-level barriers to appropriate care that can be addressed to accelerate the time to diagnosis and cure for most people with TB who access care. PPAs can be conducted using existing population-based surveys and routine programmatic data. In 2017, results from five countries implementing PPAs and two countries implementing care cascades were published.[6] Data were analysed at national and subnational levels, with results revealing key gaps in the alignment of TB care services with patient care-seeking. The results are now being used to help identify and develop interventions to address the gaps in patient care. In 2016, WHO's Strategic and

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Technical Advisory Group for Tuberculosis recommended that all countries complete PPAs as part of their priority-setting and planning processes. Implementation guidelines have been published. However, to date, fewer than 10 countries have completed subnational PPA or care cascades.[9]

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To support countries in moving toward better use of data, the global architecture of TB, including surveillance, technical assistance, and donor financing, will need to better align with the step-wise, patient-centered approach. Currently, global TB results frameworks do not monitor gaps closed along the patient pathway or specific health interventions optimized to the patient experience. To addresss this, PPA need to be routinely deployed as ~~as an~~ key components of a package of evidence that informs priorities and donor assistance. While it follows that realignment of resources with patient-seeking behavior should improve the allocative efficiency of NTP's resources, further research is warranted to validate this.

1.1.3 Network optimization and big data analytics to ensure all patients have access to services

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Network optimization is one strategy that can be deployed in high-burden countries to ensure that ~~a~~ patients presenting with TB symptoms, many of whom drop out of the TB patient pathway during the 'diagnostic phase'[10], have access to diagnostic capability. Borrowing analytic approaches from manufacturing industries, network optimization seeks to answer the following questions: What proportion of presumptive TB cases is reached by the existing TB diagnostic network? How can the network best improve coverage? What is the balance between cost and effectiveness of increasing the number of instruments, establishing better sample referral mechanisms, and introducing new tools? Even a basic analysis of imperfect existing data can reveal gaps in the diagnostic cascade that can be addressed by data-driven placement of instruments and improved referral flows.

One example of this approach comes from Lesotho, where diagnostic network mapping was deployed to analyze the NTP's testing and care cascade and inform procurement decisions. Despite a high unmet need (62% of estimated total need for testing), less than half of GeneXpert testing capacity was being used in 19 of 25 sites where it was deployed. Initially the NTP planned to procure and deploy additional instruments within the network. However, an analysis found that network capacity could be better optimized by improving referral flows and adjusting the placement of existing instruments. The analysis also identified a "sweet spot" where patient demand would make it most worthwhile to deploy point-of-care diagnostics in the future.

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While this application of network design to improving efficiencies within TB programs is in its infancy, the potential of this kind of approach—not just to diagnostic capability, but all aspects of TB programing—is huge. Big data—aggregated from routine ministry of health reports, donor-agencies operating plans, private health systems, and social media as well as other sectors of government—has the potential to transform the efficiency and precision of TB programs, enabling targetted scale up of services and providing unprecedented situational awareness and analysis capability to ministers of health and NTP managers.

In the future such data systems could also provide user-friendly 'dashboards' at each level of the health system, with a single interface for both static and real-time analysis of complex systems. The use of big data will demand specialized equipment, interoperability standards, coherent data collection and analysis systems, as well as regulatory oversight.[11] However, such approaches are being successfully deployed to address other complex health system problems in the US[12,13] and elsewhere.[14] There is no reason to presume that they could not be successfully employed to help close delivery gaps for TB programs.

1.1.4 Quality management to ensure high quality service delivery

In addition to PPA and network design analyses to ensure *access* to services for all patients presenting with TB, steps must also be taken to improve the *quality* of care that patients receive when they seek care. Unfortunately, cascade of care analyses show large gaps in quality for both adults and children and both drug-susceptible and DR-TB, in many high-burden countries.[15-17] Quality management, defined by WHO as "all activites that determine quality policy, objectives, and responsibilities, offers a framework to address low-quality care enabling a process of quality improvement(QI) to achieve health outcomes".[18]

Traditionally, programmatic impacts and outcomes have been defined primarily by epidemiological measures. Such a focus, however, overlooks that outcomes tied to closing gaps along the care cascade are more relevant operationally and can accelerate progress. Quality management tools can enable front-line providers and NTP managers to address those gaps, in order to improve care quality as well as the drivers of ongoing TB transmission.

Rationale for applying a Quality Improvement approach to TB programs

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Over the past few decades, HIV programs in sub-Saharan Africa, the Caribbean, and Asia have implemented quality management (QM) programs to optimize the use of limited resources available from governments and donor agencies.[19] The basic elements of QM~~quality management~~ include a formal QM plan, a technical working group or committee, a set of performance measures, expectations for implementing quality improvement (QI) activities, staff capacity building, and patient/community involvement. These elements are necessary to achieve sustainability in the face of expected staff turnover and environmental changes that affect the stability of healthcare organizations and the workforce. Leveraging a continuous cycle of improvement involving four steps ('plan-do-check-act,' or PDCA), these programs have driven substantive change by developing local solutions to generate improvements in the quality of HIV/AIDS care. Improvements have been demonstrated across different facets of care, including treatment adherence,[20] reducing mother-to-child transmission of HIV,[21] pediatric services,[22] enhancing fidelity to treatment guidelines,[23] and strengthening clinical capacity of front-line providers.[24]

There is growing recognition of the need to employ similar approaches to improve the quality of care for patients with TB, while also enabling increased levels of accountability at all levels of NTPs. Case studies 1-3 provide examples from the public and private sector, at facility and regional level, of how QI approaches have been deployed to improve TB outcomes.

Using the cascade of care as an organizing framework, NTPs can measure quality at the facility-level using a common set of indicators that represent key steps in the care cascade and/or reflects the ~~the~~ International Standards of TB Care[25], (Table 1.1.3). National reporting of these quality indicators can help NTPs to identify low-performing facilities that may require more support or resources. Furthermore, health facilities can use the tools of root cause analysis to identify specific barriers, generate ideas for addressing the barriers, and assess whether changes lead to improvement in the quality indicators. Continuous cycles of measurement and testing (PDCA) lead to changes that can then be adopted to achieve lasting improvement. Ideally, the PDCA process involves frontline providers as well as TB survivors and their advocates to identify patient-centered solutions that can overcome barriers to delivery of high quality TB care. Ongoing monitoring of facilities and their QI work becomes a fundamental part—the quality management component— of subnational and national TB programs.

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However, as pointed out by the Lancet Global Health Commission on High Quality Health Systems, improving quality will require system-wide action, that goes beyond facility-based QI efforts (Kruk M et al, Lancet Global Health, 2018). This includes better governance for quality; adopting competency-based clinical education and training in ethics and respectful care; and creating demand for quality in the population to empower people so they can hold systems accountable and actively seek high-quality care.

Implementing a QM program -Implications for national and global stakeholders

- **National implications** - For TB programs to be strengthened by QI, it must be built into existing policies and infrastructure. To achieve this aim, quality management programs must become part of NTPs, and ideally integrated into existing national quality management programs. Ensuring that NTP managers and their teams have access to this expertise will facilitate the development of quality management activities, including measurement and improvement initiatives. A culture change in how TB data are used to improve care must occur at every level of the health system.
- **Global Implications** – There is increasing international recognition that a quality management program embracing improvement methodologies can be a powerful lever to improve donor-recipient accountability, and enhance donor efficiency. WHO has a crucial role to play in supporting a quality management agenda and creating a global culture that supports QI and accelerates dissemination of learning through peer exchange. Linking donor support to quality indicators could also improve efficiencies in donor financing and enhance transparency.

1.1.5 Recommendations

1. Quality management should become an integral part of all National TB Programs.
    a. QM requires awareness of gaps in quality, requiring regular assessment of standardized indicators to monitor the quality of TB care and services at the facility-level.
    b. QM programs should engage provincial/district health teams to oversee the measurement and improvement of TB care at facilities in their jurisdictions.
    c. National TB quality management programs should be aligned and integrated with national quality programs where they already exist.

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d. Lessons from TB QM programs need to be shared widely, to create a global culture that supports QI and accelerates dissemination of learning through peer exchange through international platforms for knowledge management.

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2. At the global level, the WHO TB report must go beyond coverage indicators and include quality indicators. Funding entities need to support scale-up of QI and require it as an integral facet of progress reports.

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1.2 Prioritize active case finding

In addition to targeting resources and analysis to ensure high quality, patient-centered care for known cases presenting for care, the second priority TB programs need to address is finding individuals with TB in high-risk populations, who have not yet presented for care. Crucial to preventing ongoing transmission, NTPs should invest in strategies to identify and diagnose these individuals through active case finding (ACF) before they present to health facilities.[26] ACF must occur hand-in-hand with scaling up access to preventive interventions. Here we discuss first the importance of ACF and then in Section 1.3 we highlight the importance of prevention interventions. Nonetheless, these two strategies—*screening and prevention*– should be programmatically inseparable and budget allocation decisions should not divorce the two.

1.2.1 Implement targeted case-finding strategies

The goals of ACF are the same in each setting: diagnose TB early and promptly initiate treatment; reduce mortality, morbidity, and costs to the patient; and reduce onward transmission. ACF strategies focused on key populations have been critical to TB control responses in low-incidence, high-resource settings, such as Europe and North America.[26] Unfortunately, such targeted solutions have not been widely implemented in most high-burden country, for lack of funds, political will, and scientific consensus[27-29]. Nonetheless, recent clinical research,[30] mathematical modeling[31,32] and considerable programmatic experience[32,33] suggest that these strategies can be taken to scale and have an epidemiologic impact. Better funded operational research is warranted to determine how to implement cost-effective, scalable strategies to find those with TB in marginalized, high-risk groups, because often they have the least access to affordable, high-quality health care.

Commented [MOU7]: Need to reference Fox, NEJM 2018 - demonstrating value of ACF in household context

This strongly links to concept of family centered rather than person centered care.

TB is one disease where the shift to family or household centered is well justified

1.2.3 Integrate efforts where possible

Incorporating TB screening into routine health services for high-risk groups, such as individuals living with diabetes or HIV/AIDS, chronic respiratory disease clinics, and prison and refugee health care programs, is widely accepted and, in many cases, recommended by WHO.[34] Such integration is preferable for high-risk groups already served by vertical, clinic-based programs. In addition, for many high-risk populations—such as those living in slums, homeless people, and people living in remote areas—new, innovative community-based case-finding strategies must be developed and implemented.

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1. 2.4 Anticipate costs

Scaling up active ACF strategies will require substantial additional resources. Furthermore, if these activities as expected identify more TB cases, the number of patients requiring treatment will increase in the short term. However, a subsequent reduction in the risk of transmission could be highly cost-effective if major and durable reductions in TB incidence and prevalence are achieved. Planning tools, such as the WHO's online ScreenTB tool,[35] can help NTPs plan their case-finding activities and prioritize risk groups for screening by modeling the potential case yields and costs of different screening approaches. The ScreenTB tool allows the user to select risk groups of interest and compare estimates of the yield of screening (including true-positive and false-positive cases found), the total costs, and the cost per case detected across the selected risk groups and across different screening algorithms.

1.2.5 Leverage technology to improve efficiency of case-finding strategies

Increasing opportunities to leverage technology may enable TB programs to overcome some challenges that have precluded widespread adoption of case-finding strategies to date. Use of anonymized, aggregated data, sourced from the health system, social media, and the private sector, has the potential to enhance both the precision and efficiency of screening interventions. Mobile, automated, digital chest radiography units, to detect lung lesions ~~with computer-assisted detection~~ in people who are relatively asymptomatic[36,37] may also help detect many more patients with infection than is possible through passive case finding or self-reporting. Point-of-care diagnostics, such as those discussed in Section 2, are also likely to transform screening campaigns in the coming decade.

1.2.6 Why ACF in high-risk populations will never be enough

ACF alone will be insufficient to eliminate TB in high-risk populations. Even if we identify more TB cases in at-risk populations, those patients will return to high-risk pools if social determinants remain unaddressed. One potential tool for highlighting TB risk inequality is the TB risk inequality coefficient (Gomes, MGM, In press, 2018), which can be employed at the national level to compare levels of TB incidence across populations and regions and highlight heterogeneity in TB risk across communities[38]. Used in combination with surveillance infrastructure and other indices of TB risk factor prevalence, such as air pollution and under-nutrition, such tools can also be used to inform multisectoral accountability framework (discussed further in section 4).

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1.2.7 Finding cases in lower-risk populations

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330 Reaching the general population through ACF should remain a low priority until high-risk populations are
331 successfully covered, not least because the evidence to support such strategies in high-burden countries
332 is sparse. Reaching the general population will, in contrast, generally be best undertaken by
333 strengthening capacity within the general health system. As such access to primary care services is
334 imperative and underscores the importance of a broader UHC agenda as part of the enabling
335 environment to end TB.
336
337 **1.2.8 Recommendations**
338 • Reach high-risk populations, beginning with those that are most easily identified. This will
339 involve active case finding (and treatment); and in particularly high-risk populations, it will
340 involve mass preventive therapy.
341 • Integrate TB screening programs into other vertical health programs as standard of care in high-
342 burden countries, ensuring tht all pregnant women, HIV-infected individuals, and people living
343 with diabetes are routinely screened for TB, and that all child health programs ask about adults
344 with TB in the household.
345 • Partner with other sectors (e.g., prisons, mines, schools) to improve access to screening
346 interventions.
347 • Engage with civil society groups to better understand the expectations and concerns of high-risk
348 groups when planning and implementing TB screening activities. This dialogue needs to be at
349 the facility level as well as the broader planning level.
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1.3 TB prevention

TB prevention is a crucial but neglected component of global control of the epidemic. For the past 50 years, strategies for controlling TB have focused on finding and treating active cases. However, mathematical modeling shows that this approach alone, while averting deaths and relieving suffering, will not end TB. Rather, ending TB will require multiple different preventive interventions to interrupt transmission, treat latent infections, immunize close contacts, and treat or prevent comorbidities, such as HIV, that increase susceptibility to developing active TB. Figure 1.3.1 (previously 1.5.1) illustrates some populations that may benefit from prevention interventions.[39] Passive TB case finding forms the basis for global TB control strategies of the past half-century, but prioritizing active case finding is required to substantially reduce transmission by finding and treating infectious individuals before they spread infection to their close contacts. Infection control in healthcare facilities and congregate settings such as prisons is also critical: healthcare centers and hospitals are hot spots of TB transmission, and instituting rigorous policies for administrative control and use of personal protective equipment reduces rates of infection.[40]

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1.3.1 Targetted prevention interventions

TB preventive therapy (TB PT) offers one of the most important opportunities to reduce TB incidence globally. More than two-thirds of all TB cases arise in people with latent TB infection. While diagnosing and treating active TB cases can reduce onward transmission, it does not address the large reservoir of latently infected people (~2 billion) who will continue to progress to active disease in the years to come.[41] Because treating all people with latent TB infections is not feasible, targeting TB PT to those most likely to progress to active TB is essential. Clearly targeted TB PT could significantly reduce rates of TB disease in the highest risk groups, notably people with HIV infection, household contacts of active TB cases, individuals living in congregate settings such as prisons, and healthcare workers exposed to TB in the workplace. Moreover, the process of providing TB PT will uncover active cases, as candidates for PT undergo screening to rule out disease before beginning treatment, which identifies previously undetected cases of TB disease.

Although the effectiveness of TB PT is well-established,[42] public health programs have prioritized TB case finding and treatment rather than implementing this inexpensive and highly effective intervention. HIV programs have focused primarily on rolling out lifesaving antiretroviral therapy. However, recent studies have shown that TB PT using isoniazid significantly reduces rates of death in people with both early and

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advanced HIV infection. [43,44] [45] People with HIV and household contacts of active TB cases can benefit substantially from TB PT. Globally, modeling studies find that wider uptake of TB PT, coupled with improved case-finding and treatment, is more important than an effective vaccine for reaching TB elimination by 2050[45], and that household contact evaluations with PT would avert 120,000-125,000 deaths per year in children <15 years (Unpublished, Dodd). Several studies have shown that treating these high-risk individuals with novel short-course regimens to prevent TB save costs in addition to preventing illness and death.[46]

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Several obstacles have hindered the scale-up of TB PT, and innovative approaches must be taken to overcome these impediments (Table 1.3.1, previously 1.5.2). Improved diagnostic tests to document TB infection, including point-of-care (POC) tests, would facilitate treatment of household contacts, though children <5 should be treated regardless of proved infection, and all HIV-infected individuals living in high-burden areas can be treated without testing. Prognostic biomarkers that identify latently infected people who are most likely to progress to active disease would allow broader use of PT in lower-risk populations. Global supplies of essential drugs such as isoniazid (INH) and newer agents such as rifapentine are unreliable, and stock-outs are frequent; improving the supply chain of inexpensive and quality-assured products is critical. The duration of PT using INH, now 6-9 months, often results in non-adherence and is costly; novel short-course regimens, such as 12 weeks of rifapentine and INH, or a 4-week regimen of rifapentine and INH, could transform preventive efforts. [46-48] Unfounded concerns that PT will result in drug resistance have also hampered uptake.[49]

To realize the full impact of this intervention, NTPs must devote resources to ensuring that ACF and TBT are integrated into existing vertical programs for specific high-risk populations, such as child and maternal health services, diabetes clinic and primary care services for refugees and migrants, and occupational health servies for helalth care workers and miners. Integrating TB screening and preventive services into care for ~~for~~ PLWH is particularly important, especially given extensive, high-quality research demonstrating the life-saving benefits of this strategy.[43,50] Global efforts to provide antiretroviral therapy have now reached 20 million individuals with HIV, but another 17-19 million remain untreated. Fewer than 4 million people with HIV have ever received TB PT, however, highlighting the opportunity to substantially scale-up this intervention. Failure to scale up TB prevention in people with HIV has likely caused several million deaths over the past decade.[50] Further, failure to scale up access to TBT for PLWH is likely to continue to lead to substantial loss of life over the coming decade.

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Conducted in collaboration with the Lancet Commission, a team at Imperial College, School of Medicine and xx conducted an analysis to determine the impact of isoniazid preventive therapy in countries with high rates of TB/HIV co-infection. By increasing TBT among PLWH in Kenya to 90% [Figure 1.3.12], TB mortality could be reduced by 33% between now and 2045. In South Africa, a similar increase in TBT coverage, would lead to an even greater reduction in mortality over the same time frame. In addition, the incremental cost to the TB programme would be relatively modest in both countries – US$66 million per annum between 2018 and 2045, especially when compared to the economic costs of avoidable deaths resulting from failure to implement this strategy.

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To date, TB control programs have prioritized passive case-finding and treatment while neglecting to evaluate even the highest risk household contacts (childen <5 years old); new WHO guidelines, however, recommend treatment of all household contacts with latent TB, regardless of age. To accomplish this, primary health services and maternal-child health programs will need to evaluate household contacts and provide TB PT to those with HIV, <5 years old, or infected with latent TB. As shown in Table 1.3.2 (previously 1.5.2), shared responsibility for providing TB PT across the health system is necessary to reach the populations at greatest risk. Other populations at higher risk of TB that can be served by primary health services include people who inject drugs and healthcare workers in high-burden areas.

Current progress towards ending TB is woefully inadequate, with incidence rates declining less than 2% per year. Mathematical modeling suggests that substantially scaling up TB PT can greatly accelerate rates of decline in TB incidence over the next 20-30 years. Clearly the status quo guarantees millions more TB deaths in the coming decades. A vigorous effort to prevent TB cases and deaths by treating those with latent infection who are at the greatest risk of becoming ill is essential to control and end the TB epidemic within a generation.

1.3.2 Recommendations

Ending TB will require scaling up multiple different preventive interventions including treating active cases, TB preventive therapy for those at highest risk for developing active TB, and treating or preventing comorbidities, such as HIV, that increase susceptibility to developing active TB.

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446 • Targeting TB PT to those most likely to become sick must be implemented with the same level of
447 effort as finding and treating the millions with active TB disease.
448 • To fully realize the impact TB PT can provide, shared responsibility for providing TB PT across the
449 health system is necessary to reach the populations at greatest risk. TB PT should be integrated
450 into the primary health services for patients at high-risk of becoming sick, including children,
451 close contacts of active cases, health workers, and people with co-morbidities, such as HIV or
452 diabetes, that make them more vulnerable to TB.
453 • TB PT must be scaled up in all people with HIV: they should be screened for TB and provided TB
454 PT if not infected, and undergo annual screenings thereafter.
455 • Hot spots of TB transmission—including healthcare centers, hospitals, and congregate settings
456 such as prisons—must institute rigorous policies for infection control and the use of personal
457 protective equipment to reduce the spread of TB in these settings.

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1.4 importance of private provider engagement: from acknowledgment to prioritization

In most low- and middle-income countries, private providers are an important source of healthcare for people of all socioeconomic groups. Strictly speaking, "private" is synonymous with "non-state" and includes the for-profit as well as the non-profit sectors, i.e., non-governmental organizations (NGOs) and faith-based organizations (FBOs). While most countries could improve their engagement of public and NGO/FBO providers, engaging for-profit private providers, which is even more important for TB control, has been much more difficult. In this section, we discuss some reasons for the failure to engage private providers, recent progress in how they can be engaged on a large scale for TB care, and the critical actions countries must take to prioritize private provider engagement as part of their TB programs.

1.4.1 Lack of prioritization of engaging private providers

The need to engage private providers for TB control has been acknowledged since the early 1990s.[1] Successive global strategies and plans, including the recent End TB Strategy and the Global Plan to End TB, have all recognized the need to "engage all providers." WHO has published at least 11 guidance documents since 2001 on how to engage private providers, how to advocate for their engagement, how to plan for their engagement, and how to measure progress.[2] The Public-Private Mix (PPM) Sub-Group of the DOTS Expansion Working Group, established in 2002, has met multiple times since and became a separate Working Group of the Stop TB Partnership in 2016.[3] "Public-private mix" in TB has come to refer to engaging all public or private providers not directly under the NTPs, including those in prisons, the military, the police, public tertiary and teaching hospitals, as well as in NGOs and community-based groups.

Unfortunately, NTPs and their development partners have not focused on engaging private providers in TB, and resources have not been adequate to meaningfully tackle this issue. Before the most recent funding allocation, the Global Fund to Fight ~~for~~ AIDS, TB and Malaria (GFATM), which provides 56% of international development assistance for TB, had allocated less than 5% of grant budgets to engage a range of non-NTP providers defined as part of the "public-private mix."[4] Because the GFATM responds to country requests for how its grant funds will be used, ultimately this low percentage reflects the low priority that countries place on engaging their private providers. Although data on how much NTPs spend to engage private providers is scant, an example from India is illustrative: just 1.5% of the state-level TB expenditure in 2008 was allocated to engage NGOs and private providers.[51]

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488 Failure to engage private providers is often blamed on NTP staff shortages, but clearly the constraints
489 are much more profound.[52] Most health systems in low- and middle-income countries are weak in areas
490 essential for effective private provider engagement, such as regulatory enforcement, strategic
491 purchasing, and health information systems. NTPs often lack basic information on the number of
492 private providers, their role in TB patient care-seeking, and the drivers of patient and provider
493 behaviors. Therefore, NTPs find it difficult to engage hundreds and thousands of independent private
494 providers with widely varying capabilities. For their part, private providers are often wary of engaging
495 with government programs and, given competitive market dynamics and financial imperatives, unwilling
496 to adhere to guidelines promoted by NTPs.

497 Failure to meaningfully engage private providers reflects a strong preference for the public sector
498 among those who manage TB programs, those who fund them, and those offering technical support.
499 The TB community has successfully embraced many innovations, including new diagnostics, treatment
500 tools, and approaches to address TB/HIV and multidrug-resistant TB (DR-TB). These innovations,
501 however, could be adopted without challenging the basic public sector business models. Private
502 provider engagement can succeed at scale only when NTPs acknowledge that they cannot continue
503 using the current business model (Table 1.4.1). Nonetheless, such engagement must occur in tandem
504 with strategies that protect patients and their families from catastrophic financial losses that can arise
505 from accessing care in the private sector.[53,54]

506 **1.4.2 Recent progress and new opportunities to engage private providers**

507 Although private provider engagement in TB is far from adequate, considerable experience has accrued
508 regarding how to successfully engage private providers for TB care.[7] Many small, externally supported
509 pilot projects to engage private providers have been implemented over the years. A study in 2006
510 reviewed data from 15 projects in 8 countries,[55] a systematic review in 2011 considered 45 studies from
511 22 projects in 12 countries[56], and another in 2016 found 78 studies documenting 48 programs in 16
512 countries.[57] Unfortunately, most projects have failed to reach significant scale or to be sustained over
513 long periods. Nevertheless, these projects have generated abundant evidence that engaging private
514 providers can significantly increase TB case detection and achieve treatment success rates that are at
515 least as good as those in the public sector. Data on cost-effectiveness, financial protections, delays to
516 treatment, and reaching the poor is less robust but also available.[11] New research continues to add to
517 our understanding of the functioning of private healthcare markets with respect to TB.[12-14]

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