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HIV vaccine research and development: progress and promise

Guest Editors: Margaret I. Johnston, Linda-Gail Bekker, Gabriella Scarlatti, Punnee Pitisuttithum
Supplement Editor: Marlène Bras



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EDITORIAL

HIV vaccines: progress and promise

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As the end of 2021 nears, the world continues to battle the COVID-19 pandemic. SARS-CoV-2, the etiological agent of COVID-19, has infected over 200 million individuals and claimed the lives of over 4 million of them (https://covid19.who.int/). COVID-19 continues to periodically surge in many parts of the world. Even though highly effective vaccines were identified and granted emergency use authorization in 2020, most middle- and lower-income countries have yet to obtain and distribute sufficient doses to afford large-scale protection against new infections. Furthermore, viral variants that arise during replication threaten to diminish the protective efficacy of first-generation COVID-19 vaccines [1].

Unlike HIV, most, but not all, SARS-CoV-2-infected individuals mount an effective immune response that leads to full recovery, which provided a "proof-of-concept" that an effective vaccine could be identified. Enormous financial incentives and novel funding mechanisms ensued [2]. Private companies readily took up the challenge and advanced candidate vaccines into human studies in record time. In many cases they employed platform technologies, such as recombinant adenovirus 26 and mRNA, that had been explored in the development of vaccines to prevent HIV, Ebola, influenza and other infectious diseases [3]. The scientific community, especially HIV vaccine trialists with established clinical sites, community relationships and laboratory procedures, quickly pivoted and formed new alliances to demonstrate the success of those vaccines [4].

Now, 40 years after the first report of the disease now known as AIDS, proof that an HIV vaccine is possible, has relied heavily on active and passive vaccine studies in non-human primates (NHP), the RV144 phase 2b trial in Thailand, and studies of passively administered monoclonal antibodies in NHP and, more recently, humans [5–8] The scientific challenges faced in the quest to identify a safe and effective HIV vaccine remain unchanged: enormous variability in the outer membrane envelope, which enables evasion from immune responses; a "glycan shield" that hides sites that might otherwise be vulnerable to antibody (Ab) neutralization, and the absence of financial incentives that would sway the private sector to put their full weight into the effort [9].

This special issue of the *Journal of the International AIDS Society* (JIAS) was conceived as an opportunity to take stock in progress on HIV research and development, outline novel scientific and organizational approaches that might lead to success, and learn from HIV and SARS-CoV-2 vaccine studies.

1 | CURRENT PREVENTION TECHNOLOGIES ARE NOT LIKELY TO END AIDS

Efforts to identify HIV prevention approaches beyond barrier methods have resulted in several options for populations with access to HIV testing and antiretrovirals (ARVs). As summarized by Fauci et al. [10] in this issue, these options include treatment of persons with HIV to lower virus levels below what is required for transmission; oral, injectable or vaginal ring delivered pre-exposure prophylaxis (PrEP) with potent ARVs that prevent the virus from establishing infection [10]. As with barrier methods, these interventions require consistent adherence, which is challenging in settings where stigma is prominent, or where dependable access to these interventions is not feasible due to logistical, cost or other obstacles. On-going efforts to develop long-acting ARVs or broadly neutralizing antibody (bNAb) cocktails for prevention, which if only required every 6–12 months, could somewhat ease these challenges.

Since all existing prevention methods require adherence, product developers and trialists need to design and evaluate approaches that individuals will effectively use. Acquiring community perspectives and input into product development and testing and understanding what individuals at risk are most likely to consistently use, are imperative to success. Given the wide diversity of at-risk populations, including men who have sex with men, sex workers, people who use drugs, adolescent

girls and young women in sub-Saharan Africa, providing some choice in prevention interventions will likely be required to achieve population-level impact on virus spread. As attested to by Luba et al. [11] in this issue, a safe and highly effective vaccine would be a valuable addition to the existing prevention toolbox.

2 | NEW MODELS OF COLLABORATION AND PARTNERSHIP ARE PROVING FRUITFUL

Advances made in the past 25 years have been facilitated by the creation of new organizations and collaborations designed to bring additional resources and energy into the HIV vaccine development field. One such organization, International AIDS Vaccine Initiative (IAVI), has made significant contributions over the years, as described in Feinberg [12]. Perhaps most notably, IAVI researchers collected specimens that enabled isolation of broadly neutralizing antibodies and facilitated the transition of new candidates from academic settings into the clinic [9] leading to some very exciting new antibody candidates.

A significant shift in the field took place following the 2003 Science publication calling for creation of a Global HIV Vaccine Enterprise (the Enterprise), which received support from the G8 the following year [13,14]. The G8 called for creation of an alliance of researchers from around the globe to synergize efforts, avoid unnecessary duplication of efforts, standardize laboratory measurements to permit valid comparisons among studies and promote greater capacity for the manufacture and distribution of any vaccine. This call to action inspired the formation of two new collaborative inter-disciplinary programs that focus on advancing novel vaccine designs, that is, The Center for AIDS Vaccine Immunology, now the Center for AIDS Vaccine Development and the Collaboration for AIDS Vaccine Discovery (CAVD). Notably, CAVD researchers are required to share data with others in the CAVD program prior to publication, and CAVD awards include global access requirements. About the same time, the HIV Vaccine Trials Network was expanded to include more international sites, especially in Africa and South America, which has proven key to the successful conduct of efficacy studies for both HIV and SARS-CoV-2. The evolution of these and other important collaborative efforts, including EAVI2020 and Accelerate the Development of Vaccines and New Technologies to Combat the AIDS Epidemic (ADVANCE), are summarized in Feinberg et al. [12].

The Global HIV Vaccine Enterprise also evolved over time and has served to bring together stakeholders to discuss and address roadblocks such as how HIV vaccine efficacy trials might be conducted in the face of expanded access to other prevention approaches. Tatoud and co-authors provide a valuable perspective on the past and potential future role of the Enterprise [15].

The UNAIDS has also made significant contributions to the field, most notably the creation of a consensus document on the ethical considerations in HIV prevention trials [16]. Almost all guidance points have changed as the prevention field has evolved; key changes are summarized in Slack et al. [17].

3 | WHERE DO WE NOW STAND?

The lack of an effective vaccine has not been due to a lack of effort. Over $800 million USD was spent on HIV vaccine research and development in 2019 alone [18]. To date, one study conducted in Thailand showed modest vaccine efficacy, which appeared to wane over time [6,19]. A modified version of that vaccine, when tested in a higher incidence population in South Africa, failed to show any efficacy [20]. Other advanced candidates have either failed to show efficacy or remain under study, as summarized in Kim et al. [7].

One novel vaccine design still in efficacy testing at the time of this writing is based on mosaic antigens, which were designed to afford broad coverage across HIV clades. The Imbokodo and Mosaico trials are evaluating a prime-boost regimen that combines an rAd26 vector and protein. Unfortunately, following a recommendation from its data and safety monitoring board, the Imbokodo study will not be moving forward to its second phase of follow-up due to inadequate efficacy although no safety concerns have been raised. Another study referred to as PrEPVacc is evaluating PrEP and two vaccine candidates: rDNA with gp120 boost and rDNA/gp140 followed by MVA/gp140 boost. This novel multi-arm, multi-stage adaptive trial will employ an averted infection ratio method to determine efficacy. These trials, other on-going and past vaccine studies, as well as innovative studies of bNAbs, native envelope trimers, germline targeting immunogens, mRNA and new adjuvants are reviewed in Kim et al. [7]. Most recently, Moderna has launched a first in human safety trial of a novel mRNA-based vaccine to prevent HIV (mRNA-1644) in partnership with IAVI.

Vaccine design has benefited from improved structural biology tools, as reviewed by Derking et al. [21]. Structural studies have provided insights as to how to modify the HIV envelope to expose sites that are vulnerable to neutralization, as well as how to develop stable, "authentic" looking HIV envelope trimers. Understanding how the enormous glycan shield on the HIV envelope protein masks certain sites of vulnerability has led researchers to produce immunogens designed to induce antibodies that are "on target" and avoid inducing antibodies that are "off target." Recent technical advances now enable the identification of the sites of antibody binding at an atomic level.

The sera of a small percentage of individuals living with HIV contains bNAbs, which has provided the proof-of-concept that a bNAb-inducing vaccine is possible. Detailed studies of how both the HIV envelope and the body's Ab response evolve over time in those individuals have led to a strategy that attempts to replicate that evolutionary process through sequential immunization with multiple immunogens [22]. The first step along this pathway is to devise an immunogen that activates unmutated common ancestors of the desired bNAb, followed by boosting with different immunogens designed to "mature" the response to one that includes plasma cells producing the desired bNAb. This strategy and specific examples being pursued are reviewed in technical detail by Williams et al. [23].

Schief and collaborators designed a germline targeting immunogen, eOD-GT8, that was able to bind to VRC01-class precursor naïve B cells from uninfected individuals [24].

Subsequent studies initially described at the R4P conference in early 2021 demonstrated that this immunogen could expand such cells in human volunteers [25]. This is perhaps the most promising study to date supporting the hypothesis that vaccines might be able to do better than the immune responses and induce bNAbs that neutralize HIV upon its entry into the body, or that immune memory of such responses will be sufficiently fast and durable to prevent the establishment of infection. Now comes the challenge of determining what additional immunogen(s) are required to trigger further B-cell development along the desired pathway, which could benefit from the application of mRNA vaccine technology used in COVID-19 vaccines. In addition, responses will need to be more durable and broader than the CD4 binding site targeted by VRC01.

An alternative to the induction of bNAbs through active immunization is the passive administration of bNAbs, referred to as AMP, or antibody-mediated protection. Two studies of a passively administered bNAb, VRC01, to volunteers at risk for HIV infection were completed recently. As summarized in Miner et al. [8], infusions of VRC01 administered every 8 weeks did not prevent infection overall, but VRC01 was highly effective at preventing infection with strains of HIV that were susceptible to VRC01 in vitro at the level of Ab achieved in the trial. This has led to the hypothesis that protection could be achieved following administration of mixtures of bNABs (or bivalent or trivalent bNAbs) that are sufficiently potent to neutralize a high percentage of viruses in vivo. Such studies are underway or planned. Importantly, the results of that initial VRC01 trial demonstrated that neutralization in the TZM-bl/pseudovirus in vitro assay can reliably predict protection from HIV infection in humans, thus, setting the stage for a rationale selection of antibodies that could prove highly effective [24].

4 | THE FUTURE

Despite the disappointment of Imbokodo the Mosaico efficacy study, which is being conducted in men who have sex with men and transgender people, and which employs a boost with two glycoproteins, is continuing. If Mosaico shows a modest or better level of efficacy, then hope remains that induction of antibodies that bind to but do not necessarily neutralize HIV, along with HIV-specific T cells could prove beneficial. If the results are "modest" (i.e., lower bound of efficacy estimate above zero but not sufficiently high to warrant licensure, e.g., <70%), then efforts to improve upon those results will likely fall to the corporate sponsor, governments and non-profit partners. Whether such a collaborative effort will be undertaken will likely depend on the progress made with other passive and active immunization strategies as well as the uptake of existing prevention measures. If, however, efficacy is sufficiently high to warrant licensure, then attention will need to turn to tackling implementation issues including bridging to other populations and other virus clades.

Similarly, if passive administration of bNAbs or active immunization with a series of immunogens, as proposed by Corey et al. [8] and Williams et al. [23], respectively, prove successful, practical downstream challenges will still need to be addressed. These include how to integrate those complex regimens with other prevention strategies such as oral PrEP and long-acting PrEP; how cost-effectiveness data will be considered; whether manufacturing and distribution systems are sufficient; and how public health agencies will take personal choice into consideration when deciding what interventions to support. These questions have important ramification for health care systems in both highly and under-resourced settings. How best to obtain prequalification from the World Health Organization (WHO) and a positive recommendation of WHO's Strategic Advisory Group of Experts on Immunization, the role of Gavi, the Vaccine Alliance and gathering of real-world effectiveness data will need to be tackled. These issues will require continued, novel collaborations among stakeholders, with special consideration to the views of the potential "user" community, as even the best prevention measures will fail if they are not only safe and effective, but also available, affordable, accessible and reliably utilized.

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DISCLAIMER

LGB is on the Expert Advisory Group of the Global HIV Vaccine Enterprise.

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VIEWPOINT

Fitting a vaccine into the HIV prevention landscape

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The development of highly effective treatment and prevention strategies for HIV has been a significant scientific achievement over the past 25 years. Although progress has been made in the global access of antiretroviral treatment (ART), the UNAIDS 2020 global targets were not achieved because of structural and individual barriers. Therefore, the global targets are being revised for the 2025–2030 timeframe [1]. In this article, we will discuss the current state of HIV treatment and prevention and describe the research agenda that will help advance the next generation of strategies. In the context of this rapidly evolving prevention and treatment landscape, the levels of efficacy expected from an HIV vaccine increase. While using some of the lessons learned from SARS-CoV-2 vaccines, we will also point out that a safe, efficacious and durable HIV vaccine is achievable and remains a worthy goal.

As of June 2020, global ART coverage was estimated at 26 million people living with HIV (PLWH), two-thirds of the universal treatment target. Current estimates of adherence suggest that 59% of PLWH are virus-free worldwide and thus can expect relatively normal life spans [2]. Furthermore, global endorsement of "undetectable equals untransmissible", also known as U = U, makes ART the most potent and widely used preventive approach today [3]. The first long-acting injectable ART regimen delivered via monthly injection has been approved by regulatory authorities. Additional long-acting formulations are in development, including several containing broadly neutralizing antibodies (bNAbs) that could potentially extend the injection time from once every 6 months to once a year. Simplified delivery of long-acting combinations will result in both greater population coverage and durable virologic suppression. Furthermore, greater population coverage with these potent, durable regimens will boost the role of treatment as prevention in helping to control the global HIV pandemic. With these significant periods of time between doses, long-acting ART may well soon become a preferred option for treatment of PLWH.

Except for barrier methods and adult medical male circumcision, all successful HIV prevention methods rely on antiretroviral drugs [4,5]. The hypothesis has been that if there is a sufficient concentration of drug blocking HIV replication in the exposed tissues, then virus cannot establish infection, allowing the individual to remain HIV-free. Studies of different drug regimens of oral pre-exposure prophylaxis (PrEP) have shown that with adherence, protection from acquisition of HIV is remarkably high, greater than 95% and consistent across populations [6]. However, with only approximately a million PrEP users in 70 countries, the full impact of this powerful preventive tool is far from being fully achieved [6]. A major challenge facing PrEP-based HIV prevention has been the lack of sustained use by individuals who would gain the most benefit from the intervention. In studies comparing methods to improve PrEP rollout, high PrEP uptake and initiation was achieved at onset, but was then followed by significant levels of PrEP discontinuation [7]. One strategy to address adherence challenges has been the development of long-acting PrEP. In 2020, PrEP trials of an injectable drug, administered every 8 weeks, demonstrated superior efficacy compared to the standard daily oral PrEP among at-risk women and men. This significant achievement will soon lead to drug approval and implementation [8,9]. As with therapy, new agents for PrEP are moving forward in the evaluation process that could increase the amount of time between doses to once or twice a year. This could lead to increased adherence, with significant improvements in effectiveness and less burden on the health system, compared to daily pills.

In addition to antivirals, bNAbs are being explored as prevention modalities. The recently completed Antibody Mediated Prevention (AMP) trials demonstrated that the protection from infection is governed by the sensitivity of the virus to the bNAbs [10]. In addition to advancing important options for treatment and prevention of HIV infection, bNAbs also serve as a window into the levels of activity a future HIV vaccine will need to be able to trigger [11].

New medications for HIV treatment are also being evaluated for HIV prevention. Based upon the properties of each new drug, a variety of dosing options are under consideration – from weekly to once or twice a year, delivered by pill or injection. Currently, biomedical HIV prevention modalities

include daily pills, monthly vaginal rings and injections every 8 weeks. The next generation of bNAbs have also been engineered to have optimized neutralization breadth and longer half lives *in vivo* [12]. Optimized cocktails of two and three antibodies are in development and, based upon the AMP results, have a high probability of success.

Using bNAbs as a guide, it is reasonable to set an aspirational goal for vaccine efficacy that is significantly higher than previously proposed. Progress is being made in defining the requirements for induction of bNAbs via immunization and using new tools and technologies to deliver specifically engineered vaccines it should be possible to reproducibly and durably trigger production of antibodies from at least three bNAb families. Based upon the activity of bNAbs, it may be possible to achieve a level of efficacy in the 75–85% range.

Importantly, there are two HIV vaccine efficacy trials ongoing, HVTN 705, Imbokodo, and HVTN 706, Mosaico [13,14]. As an approach to facilitate and improve HIV vaccine discovery, there are lessons we can learn from SARS-CoV-2 vaccines [15]. As research focused on HIV vaccine discovery moves forward, there are many new tools and platforms we can adapt from the SARS-CoV-2 vaccines, including the utilization of various platform technologies and the development of optimally conformed immunogens. Acknowledging that the natural immunity that is induced by current HIV vaccines or HIV infection does not provide protection, the new tools that SARS CoV-2 can provide help to iterate vaccine strategies faster [15,16]. Addressing the fundamental challenges of HIV vaccine development, combined with operationalizing new concepts, and fostering significant industry engagement will be the research focus for the next decade. The research described throughout this Journal Supplement will play a critical role to achieve our 2030 targets of durable control of the HIV pandemic.

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VIEWPOINT

HIV prevention today: do we still need a vaccine? A community perspective

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Despite tremendous advances made in the global HIV response, overall progress in HIV prevention efforts remains too slow to reach the 2030 targets [1]. In 2019 alone, 1.7 million people acquired HIV, three times more than the UNAIDS 2020 target [2]. About 62% of the new infections were among key populations, these are men who have sex with men, sex workers, transgender people and people who inject drugs, and their sexual partners, and one in four new infections in sub-Saharan Africa are among adolescent girls and young women [2]. Ending HIV as a global health threat will require rapid scale up to near universal coverage of high impact HIV prevention and treatment interventions [3].

Thanks to research and development, more HIV prevention tools are now available or in the research pipeline. However, the current tools remain inaccessible to the majority of those who need them either due to access challenges; their cost, availability, structural barriers to access as well as challenges with their use; adherence, and personal choice and preference. Therefore, it is believed that achieving universal coverage of high impact HIV prevention tools will still require developing additional tools, including a safe and effective vaccine.

Since the approval of the first oral HIV prevention pill, it has become commonplace to hear declarations that we now have the tools we need to end HIV. These statements discount access, uptake and preference challenges. In the last decade, we have added oral, vaginal ring and injectable PrEP and long-acting treatment to the existing toolbox of highly effective HIV prevention strategies, but we still need a highly effective vaccine to end the global pandemic for good.

Vaccines are liberating – Most vaccines offer long-term protection against diseases [4]. The measles vaccine provides lifelong protection, and others, including vaccines for tetanus, polio and hepatitis B, provide protection for 10, 20 or more years. People who have been vaccinated do not have to think about protecting themselves from the diseases against which they have been vaccinated. Adherence and the need for regular engagement with the healthcare system becomes less of an issue.

Vaccines are easy – Healthy young people and those who do not see themselves at risk for HIV do not prioritize protecting themselves against HIV, especially if doing so requires payment and accessing unfriendly healthcare systems. An effective safe and durable vaccine would go far in meeting the needs of many different populations.

> *"Even in the absence of an effective HIV vaccine, which would be the final nail in the coffin of the pandemic, we have the tools to end the HIV/AIDS epidemic in the United States and globally". –Dr. Anthony Fauci [5].*

We agree with Dr. Fauci – "We still need an effective HIV vaccine to bring an end to the HIV pandemic". Science has shown us that developing an HIV vaccine has been quite challenging because the HIV mutates rapidly and has its own unique ways of evading the immune system. It has been over three decades since the first human trial of an HIV vaccine candidate was conducted in the United States. To date, no licensed vaccine exists, but multiple clinical trials are underway to find an effective HIV vaccine.

The story is different for COVID-19. Decades of HIV vaccine research and advocacy made COVID vaccines possible. In less than a year, the global COVID 19 response has produced the fastest, best coordinated and funded vaccine development effort in history, leading to the discovery of multiple highly effective vaccines in record time. The global partnerships and the political and financial commitment demonstrated in the development of COVID-19 vaccines and therapeutics should be replicated in the HIV vaccine research and development space.

Besides the critical RV144 trial, there have been a number of efficacy trials which have been stopped for futility, including the most recent Uhambo (HVTN 702 trial) [6]. Uhambo was a phase 2b study which began in 2016 to evaluate the preventive vaccine efficacy, safety and tolerability of ALVAC-HIV (vCP2438) + Bivalent Subtype C gp120/MF59 in

HIV-seronegative South African adults over 24 months and potentially up to 36 months from enrolment [7]. This trial was based on the positive results of RV144 and its cancellation has been a blow to the HIV community. There are, however, other opportunities in the pipeline.

After RV144 reported estimated cumulative efficacy of 60.5% in the 12-months after initial vaccination, Dr. Robert Gallo was quoted as saying "You could make the argument that a vaccine could be licensed if it reaches 60%" [8]. The current approved COVID-19 vaccines have set the efficacy bar very high. And now the question is, will communities accept an HIV prevention vaccine with less than 60% efficacy? Community preparedness for HIV vaccine trial results will be key. There will be a need for strong collaboration between researchers and community advocates in the provision of HIV vaccine research literacy and preparing the communities for whatever results we might end up getting.

The dosing and the timing between doses is another important key consideration. The HIV vaccine development community should draw lessons about acceptability, access and user preference on dosing and timing from COVID 19 vaccine rollout programs. These questions should also be embedded in the design of current and future HIV vaccine trials. Preparing for the rollout of long-acting injectable PrEP products presents potential opportunities to learn about what an HIV vaccine rollout and preferences could look like.

COVID 19 has also exposed alarming rates of vaccine hesitancy, which is likely going to be a major challenge for HIV vaccine development and rollout, necessitating the need for robust community engagement during ideation and implementation of clinical trials as well as vaccine rollout to clear doubts, myths and misinformation about vaccine science.

HIV remains a global pandemic that kills more than 700,000 every year. Finding an effective HIV vaccine is not an option. It is a must. Development of a vaccine to prevent HIV would relax requirements for frequent visits to HIV specialists, pharmacy and laboratory services as well as reduce the associated out-of-pocket healthcare costs.

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All authors confirm that they have no competing interests.

AUTHORS' CONTRIBUTIONS

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VIEWPOINT

The importance of partnerships in accelerating HIV vaccine research and development

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Forty years since the first report of the disease in the United States now known as HIV/AIDS, an efficacious HIV vaccine remains an elusive goal. However, the remarkable level of discovery, innovation and collaboration employed in pursuit of this goal has augmented the scientific insight and capabilities of vaccinology overall and benefitted vaccine development targeting other infectious diseases. Importantly, the progress made in the HIV vaccine research effort is not just a story of the "what" but also the "how." Given the complexities that make HIV a confoundingly difficult target for vaccine development, and the lack of market opportunity that would encourage pharmaceutical companies to make substantial and sustained at-risk investments, innovative partnerships for HIV vaccine development have offered a promising path forward. Just as HIV vaccine science has provided the leading edge of vaccine development innovation, the models of collaborative science pioneered in the HIV vaccine field also represent innovations. Indeed, the science and partnership models advancing HIV vaccine development helped to dramatically accelerate the development of highly efficacious SARS-CoV-2 vaccines [1,2]. This viewpoint describes major partnerships and funding models that have driven HIV vaccine discovery and translational science.

Many collaborations have been established over the past 25 years to advance HIV vaccine research by enabling a "big science" approach. Sustained funding and a common strategic focus have enabled greater coordination, faster design and development of immunogens, and more effective sharing of data and technical capabilities.

Chronologically, IAVI (initially known as the International AIDS Vaccine Initiative) was the first of these partnerships to be established. IAVI, founded in 1996, emanated from a call for a new global initiative to accelerate the development of HIV vaccines for worldwide use [3]. IAVI and its major Neutralizing Antibody Center partner, Scripps Research, continue this mandate. HIV vaccine candidates are designed using high-resolution structure-based immunogen design techniques with the intent of eliciting broadly neutralizing antibodies (bnAbs). In addition, IAVI, Scripps Research and the Vaccine Research Center at the National Institutes of Health (NIH) are advancing combinations of optimized bnAbs as a potential passive immunization tool with the potential for affordable global access, in partnership with the Serum Institute of India and USAID [4].

The Global HIV Vaccine Enterprise (Enterprise) is an alliance of independent funding, research and advocacy organizations launched in 2005 to mobilize new resources for HIV vaccine research and development (R&D) and promote the acceleration of promising research strategies. The Enterprise, through its first strategic plan, provided a framework that sparked the creation of other major partnerships: in 2005, the Bill & Melinda Gates Foundation (BMGF) launched the Collaboration for AIDS Vaccine Discovery (CAVD) and the NIH-funded Center for HIV/AIDS Vaccine Immunology (CHAVI).

The CAVD is a network of translational research consortia focused on the design and development of novel vaccine candidates. The CAVD accelerates research through the open sharing of data, and utilization of central service facilities that include best-in-class B- and T-cell immunology laboratories, sophisticated mouse models, a data and statistical management centre and a vaccine product development centre that facilitates the translation of promising concepts from the lab into clinical evaluation [5]. This highly collaborative framework has accelerated a range of complex basic, translational and clinical studies that would not have otherwise been possible for individual investigators to pursue.

The CHAVI program founded at Duke University was another multi-institutional partnership predicated on interdisciplinary and collaborative science. CHAVI investigators have studied virus transmission and elucidated many of the basic aspects of HIV immunology that now inform our mechanistic understanding of requirements for HIV prevention. Building on this effort, in 2012, the NIH funded two Centers for HIV-AIDS Vaccine Immunology and Immunogen Discovery (CHAVI-IDs) at Duke University and Scripps Research to focus on HIV immunogen design. In 2019, Scripps and Duke

Table 1. Partnerships

Partnership name	Partners	Year founded	Purpose
IAVI www.iavi.org	IAVI, Rockefeller Foundation (founding donor), BMGF, USAID, others	1996	Translate scientific discoveries into affordable, globally accessible public health solutions
HIV Vaccines Trial Network (HVTN) www.hvtn.org	NIH, Fred Hutchinson Cancer Research Center, others	1999 (The AIDS Vaccine Evaluation Group was funded by NIH in 1988 [11].)	Characterize the safety, immunogenicity and efficacy of HIV vaccine candidates
HIV Prevention Trials Network (HPTN) www.hptn.org	NIH, FHI 360, Fred Hutchinson Cancer Research Center, Johns Hopkins University, Imperial College London	1999 (The HIV Network for Prevention Trials [HIVNET] was funded by DAIDS in 1993 [12].)	Develop and test the safety and efficacy of interventions designed to prevent the acquisition and transmission of HIV
Neutralizing Antibody Consortium	IAVI, Scripps Research, NIH Vaccine Research Center (VRC), others	2002–2008	Discover, describe and optimize HIV bnAbs and develop immunogens to elicit them
Collaboration for AIDS Vaccine Discovery (CAVD) www.cavd.org	BMGF and multiple partners, see [13]	2005	Design a variety of novel HIV vaccine candidates and advance the most promising candidates to clinical trials
IAVI Neutralizing Antibody Center (NAC)	IAVI, Scripps Research, other partners	2008	Discover, describe and optimize HIV bnAbs and develop immunogens to elicit them
CHAVI	NIH, Duke	2005–2012	Undertake the immunologic research required to tackle the major scientific obstacles in the development of an effective HIV vaccine
CHAVI-ID	NIH, Duke, Scripps Research	2012–2019	Undertake the immunologic research required to tackle the major scientific obstacles in the development of an effective HIV vaccine
IAVI Product Development Center (PDC)	IAVI, BMGF, NIH, others	2013	Support investigators with translation research [14]
European AIDS Vaccine Initiative (EAVI2020) www.eavi2020.org	Imperial College London, University of Oxford, INSERM, others	2015	Advance HIV vaccine concepts that can be moved into clinical trials in a 5-year window using an experimental medicine research model
ADVANCE www.iavi.org	USAID, IAVI, CRC partners in five African countries and India, others	2016 (with prior, sustained USAID investments starting in 2001)	Support researchers in Africa and India to become leaders in global efforts to identify, evaluate and implement HIV biomedical prevention products
CHAVD www.chavd.org (Scripps) www.dhvi.duke.edu (Duke)	NIH, Duke, Scripps Research	2019	Develop and down select HIV immunogens and regimens that induce bnAb responses for clinical testing
European HIV Vaccine Alliance (EHVA) www.ehv-a.eu	Horizon 2020, INSERM, Swiss Vaccine Research Institute at the Lausanne University Hospital, others	2016	Develop platforms for HIV vaccine research and engage African scientists to prepare for clinical evaluation of EHVA vaccine candidates
Collaborative HIV Immunogen Project (CHIP)	NIH, BMGF, Scripps CHAVD, Duke CHAVD, HVTN, NIAID Vaccine Research Center, IAVI	2019	Coordinate groups developing HIV vaccine candidates designed to lead to the production of bnAbs [9]

Abbreviations: ADVANCE, Accelerate the Development of Vaccines and New Technologies to Combat the AIDS Epidemic; BMGF, Bill & Melinda Gates Foundation; CHAVI, Center for HIV/AIDS Vaccine Immunology; CHAVI-ID, Centers for HIV-AIDS Vaccine Immunology and Immunogen Discovery; CRC, clinical research centre; INSERM, Institut national de la santé et de la recherche médicale; NIAID, National Institute of Allergy and Infectious Diseases; NIH, National Institutes of Health.

each developed Consortia for HIV/AIDS Vaccine Development (CHAVD), aimed at supporting collaborative research to translate innovative HIV vaccine immunogens into clinical development [6].

The European AIDS Vaccine Initiative (EAVI2020) and the European HIV Vaccine Alliance were established in 2015 with the goal of fostering the development of an effective vaccine. While the European Community previously funded smaller vaccine consortia, these new efforts brought together leading vaccine research groups from academia and industry across Europe. They supported new approaches that bridge laboratory and clinical research studies via iterative vaccine design and optimization programs with the goal of reducing risk of failure in late-stage development.

ADVANCE (Accelerate the Development of Vaccines and New Technologies to Combat the AIDS Epidemic), a program initiated in 2016 via cooperative agreements between IAVI and the USAID through the United States President's Emergency Plan for AIDS Relief (PEPFAR), has advanced HIV vaccine research and development through partnerships in Africa and India. The foundations of ADVANCE emerged from earlier sustained investments by USAID to establish a network of translational, epidemiological and clinical research centres (CRCs) in five sub-Saharan African countries and India [7]. Data generated from CRC-driven epidemiological studies have provided important insights into the process by which bnAbs develop in individuals with HIV — work that is foundational to the design of vaccines aimed at inducing bnAbs for HIV prevention [8,9].

Table 1 summarizes a variety of partnerships formed to advance HIV prevention research.

Many of the most effective partnerships for HIV prevention research have fostered multidisciplinary science, linking immunology, structural biology, protein science, virology, genetics and expertise in monoclonal antibody discovery and optimization. This collaboration among disciplines is possible only when intra- and extra-institutional barriers are overcome to allow unfettered cooperation, access to scientific core infrastructure and data sharing frameworks to support and connect researchers who, absent these partnerships, are typically more siloed.

Clinical trial networks, such as the HIV Vaccine Trials Network (HVTN) and the HIV Prevention Trials Network, along with other clinical development activities funded by USAID and the European and Developing Countries Clinical Trials Partnership, have enabled the conduct of clinical trials among those most at risk of HIV acquisition. These networks, most specifically HVTN, have developed clinical, laboratory and statistical standards and procedures that advanced the field and, in many cases, allowed the comparison of results from different HIV vaccine studies.

A discussion of partnerships must include mention of their potential complexities and pitfalls. Large collaborative efforts can tend towards groupthink and risk aversion if not actively avoided with diversified funding to generate and support new ideas. Additionally, clinical trial infrastructure established to evaluate late-stage candidates needs to be repurposed when vaccine candidates do not demonstrate efficacy. Increasingly, the field is focusing on higher through-put of early-stage clinical candidates to diversify the pipeline, which will require a selection process that accelerates the most promising concepts. Partnerships should be expanded to include expertise in implementation, delivery and access to HIV vaccines once they are shown to be efficacious. The global disparities in access to SARS-CoV-2-vaccines [10] have highlighted this imperative.

The development of optimal partnerships for the future will require learning from the past. Partnerships formed to advance HIV vaccine research have enabled the global scientific community's rapid pivot to SARS-CoV-2 vaccine research [2]. Now, the remarkable display of collaborative science in the response to the COVID-19 pandemic could "return the favour" to help implementation of more effective partnerships to expedite HIV vaccine development. With study of how COVID-19 partnerships have succeeded, especially in linking pharmaceutical companies with the public and academic sectors, we can ensure that our partnerships are robust and resilient enough to solve the challenges that remain on the path to an HIV vaccine.

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COMPETING INTERESTS

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AUTHORS' CONTRIBUTIONS

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VIEWPOINT

A transformed global enterprise for an HIV vaccine

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The COVID-19 pandemic has shown that the global scientific community, public and private funders, as well as governments, can come together to develop safe and effective vaccines in record time. Success was in large part due to the focus on harnessing existing and new science to develop a vaccine urgently needed for global public health use. That sense of urgency, as well as proof-of-concept that a vaccine was possible, drove the rapid discovery of COVID-19 vaccines [1,2].

Scientific advances in one field are often nurtured by previous research in other fields and progress in one area can reinvigorate research and development (R&D) in another. In many ways, the COVID-19 vaccine success builds on 40 years of HIV vaccine R&D, including basic science, vaccine platforms, clinical experience and networks, and close interactions between scientists and affected communities. HIV vaccine scientists quickly pivoted, or expanded their work, to COVID-19 vaccine R&D [3]. Beyond science, funding matched the task. Pull mechanisms, which foster demand for an undeveloped product, complemented push mechanisms, which lower the cost of R&D to fund targeted development projects and transnational partnerships. Unlike COVID-19, where science was not the main challenge, HIV vaccine development remains at a discovery stage, and the creation of a new system of incentives to mirror what is unfolding in the COVID-19 response [4] could enhance the global research effort.

In this viewpoint, we revisit the early days of the Global HIV Vaccine Enterprise (the Enterprise) and explore how the COVID-19 vaccine development effort could inform a future transformation of an organization that has supported the vaccine R&D field since 2005 (Table 1), first as an alliance of stakeholders, and now as a convener hosted at the IAS – the International AIDS Society.

At the turn of the 21st century, after 20 years of mostly investigator-driven HIV vaccine research and realizing the complexity of the challenges confronting the scientific community, it became clear that a more coordinated and collaborative effort was needed to accelerate HIV vaccine development [5]. Consequently, the Global HIV Vaccine Enterprise was established in 2005 [6]. Its initial impulse was to identify gaps in research or areas in need of better collaboration, from basic immunology to conduct of clinical trials. The expectation was that a scientific plan developed collaboratively would serve as a roadmap for the scientific community and funders [7]. Much new science was produced under the plan [8], but it was not always enough to convince scientists, donors and industry that we knew enough to proceed energetically with proof-of-concept clinical trials of a variety of candidate vaccines. Largely, it was felt that the best use of existing funding was still to solve basic scientific questions, rather than empirically conduct trials.

In 2018, the Enterprise evolved into a convening enabler with a strategy to maintain momentum for scientific collaboration while orienting the vaccine community around emerging priorities in late-stage product development [9]. Many scientific and operational challenges that hindered the development of a vaccine in 2005 remain unsolved today [10]. As we emerge from the COVID-19 pandemic, and with an HIV prevention landscape evolving quickly, the times are propitious to renew the mission of the Enterprise.

A rejuvenated Enterprise can provide the impetus for making a case for more empirical research, be a place to identify and support exploration of out-of-the-paradigm approaches and a forum to connect innovators, researchers, the pharmaceutical industry, regulators, funders and communities to explore new ways of working, stimulate coordinated long-term innovative funding strategies and foster greater dialogue between funders and researchers [11].

An HIV vaccine is still needed – and still achievable [12]. New exciting HIV vaccine science continue to be produced, strengthening our conviction that we will be able to create a vaccine. But exploratory science alone will not be sufficient to develop a vaccine, and a long-term, end-to-end strategy of product development which includes a systematic and coordinated pipeline of vaccine constructs that can be selected, tested, evaluated, refined – and evaluated in parallel rather than sequentially could accelerate the development of a vaccine.

Table 1. Global HIV Vaccine Enterprise achievements and key clinical efficacy trials

Year	Achievements and key clinical efficacy trials
2003	• Global HIV Vaccine Enterprise concept first published in Science Ref. [5].
	• AIDSVAX® VAX003 and VAX004 clinical trials show no efficacy for the prevention of acquisition or for modification of HIV infection.
2004	• The G8 at the Sea Island summit endorses and establishes the Global HIV Vaccine Enterprise.
	• Interim secretariat of the Enterprise established at the Bill & Melinda Gates Foundation.
2005	• The Global HIV/AIDS Vaccine Enterprise: scientific strategic plan published in PLoS Medicine Ref. [6].
	• First stakeholders meeting held at the Wellcome Trust in London, UK, followed by a Funder's forum.
	• Establishment by the NIH of the Center for HIV/AIDS Vaccine Immunology (CHAVI) first implementation projects under the Global HIV Vaccine Enterprise.
	• Establishment by the Bill and Melinda Gates Foundation of the Collaboration for AIDS Vaccine Discovery (CAVD), second implementation projects under the Global HIV Vaccine Enterprise.
2007	• WHO-UNAIDS, AVAC and the Enterprise form a coordinating group and communication subgroup.
2008	• First executive director appointed, and secretariat established in New York, USA.
	• Enterprise launched the "New minds new ideas" to attract the next generation of investigators and technologies to HIV vaccine research (AIDS 2008 Conference in Mexico City, Mexico).
2009	• Enterprise Science Committee established to discuss the state of the field.
	• The AIDS Vaccine for Asia Network (AVAN) created following a consultation with WHO-UNAIDS in Beijing.
2010	• Publication of the 2010 scientific strategic plan of the Global HIV Vaccine Enterprise in Nature Medicine Ref. [7].
	• Enterprise, WHO, UNAIDS and the US–MHRP convene a meeting to develop recommendations on the future of RV144.
2011	• Enterprise board identifies convening, funding and collaboration as top priorities, re-iterating the concept of preparing comprehensive scientific strategic plans.
2012	• Enterprise secretariat takes up residence at IAVI headquarter in New York under a new director.
	• Enterprise launches the Timely Topics to discuss cross cutting themes proposed by investigators.
2013	• 13th and last AIDS Vaccine Conference, Barcelona, Spain – October 7–10, marking the transition to the biannual "HIV Research for Prevention" (R4P) Conference, the only global scientific conference focused exclusively on the challenging and fast-growing field of HIV prevention research.
	• Launch of the Bench to Clinic webtool.
	• HVTN 505 DNA plus protein efficacy trial stopped due to lack of efficacy.
2014	• First Research for Prevention Conference led by Enterprise held in Cape Town, South Africa.
2015	• Enterprise holds a Product Development Bootcamp after creating a guidance document on Target Product Profile for a preventive HIV Vaccine.
	• European AIDS Vaccine Initiative (EAVI2020) and the European HIV Vaccine Alliance (EHVA) are funded by the European Commission to foster a multidisciplinary approach to HIV vaccine development.
2016	• HVTN 702 (Uhambo) launched to test whether a new version of the RV144 HIV vaccine candidate safely prevents HIV infection among adults in South Africa.
2017	• Launched of the HVTN 705 (Imbokodo) trial, a Phase 2b proof-of-concept study in HIV-negative women in Africa evaluating the safety and efficacy of an experimental regimen based on a "mosaic" vaccine designed to induce immune responses against a wide variety of global HIV strains.
2018	• Enterprise transfers to the International AIDS Society (IAS).
	• Global HIV/AIDS Vaccine Enterprise Strategic Plan 2018–2023 presented at the Research for Prevention Conference, Madrid, Spain.
	• Launch of PrEPVacc, an African-led, European-supported HIV prevention study running in eastern and southern Africa from 2018 to 2023. For the first time, a study will evaluate an experimental HIV vaccine and pre-exposure prophylaxis (PrEP) at the same time.

(*Continued*)

Table 1. (Continued)

Year	Achievements and key clinical efficacy trials
2019	• Launched of the HVTN 706 (MOSAICO), a trial using products similar to HVTN 705 recruiting HIV-negative men and transgender people who have sex with men in the Americas and Europe.
	• Enterprise at the IAS convenes its first stakeholder meeting at the 10th AIDS Conference in Mexico City, Mexico, followed by a convening of EU stakeholders in Ghent, Belgium, an opportunity to engage as a region on critical issues, including political and research commitments in Europe.
2020	• HVTN 702 discontinued for futility.
	• The SARS-CoV2 pandemic lead to global lockdowns.
	• Enterprise holds online events updating stakeholders on HIV vaccine R&D, preparing the field for result of ongoing efficacy trials, supporting new research into design for efficacy trials in a fast-changing prevention landscape and advocating for sustained investment in vaccine R&D.
	• On 11 December 2020, the U.S. Food and Drug Administration issued the first emergency use authorization (EUA) for a vaccine for the prevention of coronavirus disease 2019.
2021	• HVTN 705 (Imbokodo) discontinued for lack of efficacy.

Abbreviations: AVAC, AIDS Vaccine Advocacy Coalition; NIH, National Institutes of Health; UNAIDS, The Joint United Nations Programme on HIV/AIDS; R&D, reseach and development; US-MHRP, U.S. Military HIV Research Program; WHO, World Health Organization.

Vaccine development challenges addressed collectively can lead to solutions applied collectively. Meaningful collaborations that have delivered on challenges, such as development of broadly neutralizing antibodies, must be extended to incorporate product development and evaluation. Renewed and expanded collaborations that would emulate what COVAX, the vaccines pillar of the Access to COVID-19 Tools (ACT) Accelerator and the US Operation Warp Speed have done fast and well will benefit from the Enterprise as a convener.

Looking forward, *sustainable strategic networks* should include industry, funders and, importantly work towards effective coordination among stakeholders to promote long-term strategic cooperation [13]. Such networks must include researchers worldwide as the balance of expertise and funding still leans heavily towards the global North, a dominance that was recognized as early as 2004 when the G8 provided support for a global vaccine enterprise [14]. It is vital to ensure that most-affected communities are included in the research, which would improve demand for and acceptance of vaccines. The Enterprise can also support the role of national, regional and global organizations (including WHO and UNAIDS) that have associated aims.

Finally, in addition to new and diversified investment, the Enterprise could be an attractive go-between to bring back more industries to the table. New incentives could de-risk, scientifically and financially, involvement of the private sector, which has patiently waited for the scientific community for reassurance that basic scientific problems in development of an HIV vaccine have been solved. This is probably one of the major lessons from the COVID-19 vaccine experience; public–private partnerships can be very powerful [15].

As envisaged in 2005, the Enterprise should remain a forum where partners can share their vision, build and plan collaborative efforts. To succeed, the Enterprise needs to be given the mandate and means to engage with stakeholders to deliver on a challenge with greater complexity than COVID-19 vaccine development. Building on a leadership that has been at the centre of the COVID-19 vaccine success, it is realistic that the Enterprise could emulate the COVID-19 success. As originally envisioned: "The Enterprise would be a steering, coordinating and monitoring organization with an emphais on openness, collegiality, data sharing and commitment". The early discontinuation of the HVTN 702 study (which followed in the steps of the RV144, the only vaccine efficacy study that ever showed a positive, although modest protection), a pandemic of worldwide consequences and progress in HIV prevention strengthen the case for a new beginning for the Global Vaccine Enterprise.

The challenge will be to build consensus for change in the HIV vaccine R&D field, which many have started to acknowledge is crucial, as they have learned from COVID-19 [16]. We need to make HIV vaccine research a public health priority, as was done for COVID-19. In this changing global health landscape, the COVID-19 pandemic may be the *black swan* [17] that will give birth to a transformed global enterprise for an HIV vaccine. Our collective challenge and responsibility are to find the way to realistically implement a shared vision of a world where the HIV epidemic is finally fully controlled.

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COMPETING INTERESTS

None to declare.

AUTHORS' CONTRIBUTIONS

RT, WS and JE contributed equally to the production of this manuscript.

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DISCLAIMER

The views expressed in this document are those of the authors alone and may not in any circumstances be regarded as stating an official position.

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COMMENTARY

Shifts in UNAIDS ethics guidance and implications for ethics review of preventive HIV vaccine trials

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Abstract

Introduction: A major change in the ethics framework for preventive HIV vaccine trials worldwide is the release of the UNAIDS 2021 *ethical considerations in HIV prevention trials*. This new guidance comes at an exciting time when there are multiple HIV vaccine efficacy trials in the field. Research Ethics Committees (RECs) or Institutional Review Boards are a most likely audience for these guidelines. Our objective is to highlight shifts in ethics recommendations from the earlier 2012 UNAIDS guidance.

Discussion: We review recommendations related to four key issues, namely standard of prevention, post-trial access to safe and effective vaccines, enrolment of adolescents and enrolment of pregnant women. We outline implications and make recommendations for the ethics review process, including suggested lines of inquiry by RECs and responses by applicants.

Conclusions: There have been several shifts in the UNAIDS ethics guidance with implications for HIV vaccine researchers submitting applications for initial ethics review or re-certification, *and* for RECs conducting such reviews. This review may assist RECs in a more efficient and consistent application of ethics recommendations. However, additional tools and training may further help stakeholders comply with new UNAIDS ethics recommendations during protocol development and ethics review.

Keywords: ethics; HIV vaccine trials; REC; UNAIDS

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1 | INTRODUCTION

Currently, three preventive HIV vaccine efficacy trials are underway – HVTN 705 and 706 are exploring the Janssen HIV vaccine regimen in adults [1] and an additional trial – PrEPVacc – has begun in Africa to evaluate two DNA-based regimens in combination with oral pre-exposure prophylaxis (PrEP) [2]. Other vaccine regimens are in earlier phase trials [1]. It may be several years before other preventive HIV vaccine candidates progress to efficacy trials. Such late-phase trials, enrolling populations at high-risk, require Research Ethics Committees (RECs) or Institutional Review Boards to carefully attend to plans for participants' access to HIV prevention tools, plans for post-trial access to vaccines proven safe and effective, as well as plans for enrolment of pregnant women, and enrolment of adolescents.

While not specific to review of HIV vaccine trials, it has been argued that REC review processes can be inefficient or demonstrate unjustified variations across RECs during review of multi-site trials [3]. This may be due in part to REC review capacity when faced with complex protocols, as well as uneven application of ethics guidance. Also, the expertise with which researchers/applicants pro-actively address ethics issues impacts the efficiency and quality of ethics review [4,5]. Centralized ethics review and reciprocal review models (or reliance agreements) are used in some settings to increase efficiency and harmony of REC review; however, in many settings, ethical-legal frameworks and institutional policy lead to multi-REC review.

A major development in the ethics framework for preventive HIV vaccine trials is the release of the new *Ethical Considerations for HIV Prevention Trials* (UNAIDS, 2021) with the expressed hope that these will be a "valuable resource for RECs" [6] and other key stakeholders. While some changes have been summarized [6], there has been little effort to consider how shifts in guidance might (or should) impact RECs and researchers in the ongoing ethics review process; our objective is to describe these shifts. Given that RECs and researchers have been exposed to UNAIDS recommendations for 13 years [7], we do not present recommendations in

their entirety here, rather we focus on *shifts* in this guidance. Also, we focus on trials of preventive HIV vaccines, even while trials of other HIV prevention products are underway in order to expand the range of options for all at-risk populations [8–10].

2 | DISCUSSION

2.1 | Standard of prevention

As highly effective oral PrEP has become increasingly available, the "welcome but difficult" challenge of reduced incidence in efficacy trial design has been underscored [11]. Long-acting PrEP, which includes injectables and vaginal rings, may also become progressively more available [9] as effective products receive regulatory approval and novel drugs progress through the pipeline. HIV vaccine trial designs need to balance ensuring participants' access to prevention methods while simultaneously supporting efficacy assessments through sufficiently powered studies [9]. How to best achieve this balance has been the subject of intense discussion and debate [12].

The UNAIDS 2021 [13] revision is expressly rooted in the above dilemma, namely the need to research future interventions to address the unmet needs of at-risk populations, while simultaneously facilitating access to effective interventions for current participants. A marked change in the 2021 recommendations is that researchers should ensure that participants get access to "*WHO-recommended*" prevention methods, as opposed to "*state of the art*" prevention [7] – the latter being criticized as "infeasible in practice" [14]. Another change lies in *explicit* guidance that researchers can deviate from the "WHO-recommended" standard only for compelling scientific, biological or manufacturing reasons; and that relevant stakeholders must be meaningfully engaged in review of the deviation, and their acceptance of the deviation is required. This replaces *implied* guidance that researchers could (presumably) deviate from the "*state of the art*" standard after consultation with stakeholders taking into account feasibility, impact and ability to isolate the modality being tested. Another shift is that deviations from WHO-recommended prevention methods must be approved by RECs, which makes RECs central to decisions about acceptable prevention standards, as encouraged by previous scholars [15].

In terms of review of *standard of prevention*, RECs may establish a requirement for local applicants to demonstrate that the "WHO-recommended" standard is met for participants *at their site*, with support from networks where possible, for example, in a participant HIV prevention plan. Networks (or pharmaceutical companies) could implement engagement efforts at an international level to debate justified deviations (to decrease pressure on individual sites to achieve such engagement goals), and describe these engagement efforts in master protocols. Sites could be encouraged to develop site-specific descriptions of their local engagement efforts. RECs can conduct an efficient three-part assessment: 1) Has the standard been met? 2) If not, is the deviation justified? 3) Have engagement efforts been "meaningful"? Here, RECs can ask insightful questions regarding the quality of engagement, for example about the inclusiveness and diversity of engagement [16].

2.2 | Post trial access

Commentators have noted that "the possibility of an effective (HIV) vaccine is now tangible" [11]. COVID-19 provides sobering lessons about post-trial access to vaccines [17], including that low- and middle-income countries which take part in trials but cannot make early monetary investments will likely lag behind those countries that can invest substantially in developing and manufacturing vaccines, and can negotiate purchasing well before clinical trials and manufacturing are complete [18]. COVID-19 also underscores the need to invest in infrastructure to deliver vaccines with demanding cold-chain requirements [19,20].

A significant shift in the UNAIDS 2021 [13] guidance is that plans for access should be in the protocol. In terms of *non-participants* having access to products shown in the clinical trial to be safe and effective, the 2021 guidance reiterates earlier recommendations for sponsors and researchers to initiate discussions as well as secure an agreed plan regarding how products will be approved, paid for, manufactured and delivered/distributed, while introducing that plans considers the potential complexities of the pathway. Like its predecessor, the 2021 guidance recommends that sponsors and researchers cast the net widely for planning and discussions but 2021 guidance encourages for the first time "special attention" to populations often excluded from research and product access. In terms of access to products *by participants*, the former document made a procedural recommendation that sponsors *secure agreement* on plans to make the intervention available to participants, while 2021 guidance makes a substantive recommendation that sponsors should ensure "ongoing provision" and "continued access" to participants. It is also more nuanced than its predecessor insofar as it provides reasons for deviating from this stance. In terms of access to results, 2021 guidance introduces that *participants*, *local communities and national governments* should receive results "before or contemporaneously with international dissemination".

In terms of review of *access to products*, RECs can require protocol submissions to include statements that show planning for access to proven products (and to results) for key groups like participants, the community engaged in the trial or population in which the product was tested. In phase 1/2 protocols, RECs can inquire about potential subsequent trials as well as broad plans for access, and prompt sponsor/investigators to provide descriptions about issues, such as cost, and capacity needed to produce and deliver the product. Such inquiries relatively early on may help trigger actions with long time-frames, such as facility development to manufacture vaccines, or manage the cold-chain. In phase 3 protocols, RECs should expect more detailed planning during initial review and make regular inquiries about progress on this issue during annual reports/re-certifications. RECs can address any concerns about access to products being an "undue" inducement by ensuring careful framing in consent materials, and ensuring that study risks are not minimized or discounted [21].

2.3 | Enrolment of adolescents

Adolescents are at high risk of HIV infection worldwide because of various behavioural, biological and structural factors; however, their inclusion in trials is made complex by several elements, including regulatory frameworks [22,23]. The 2021 guidance no longer has a guidance point specifically devoted to adolescents; instead, recommendations specifically relating to adolescents are included largely under the "fair selection" guidance point, with additional remarks under "informed consent" and "confidentiality and privacy". Hence, users have to work harder to locate scattered recommendations.

In terms of adolescent-specific guidance, the 2021 document retains the 2012 recommendation to seek parental consent "unless exceptions are authorized by national legislation" but somewhat softens the stance by permitting exceptions authorized in "national guidelines". The guidance now acknowledges that parental consent for sensitive research can act as a "barrier" to enrolment; can cause "social harms" like parental sanctions; and can "skew enrolment" towards low-risk adolescents. In terms of adolescent-relevant guidance, a subtle but striking shift is that the 2021 document squarely faces the problem of under-representation of key groups in trials leading to health disparities. The revised guidance repeatedly encourages researchers to avoid arbitrarily excluding persons, that is without good scientific reasons, justifications or goals. REC members will rightly remember several 2012 recommendations along these lines, but less emphasis on the *problem* of an inequitable evidence base for under-represented groups. Also, the 2021 document attempts to shift away from vulnerable persons towards *contexts in which people live* that increase their vulnerability [6] while retaining most of the recommended responses from the earlier document. Another shift in the 2021 guidance is that researchers are encouraged to recognize gender diversity; to include gender-diverse groups in trials; and to attend to the needs of individuals of all gender identities and expressions.

The 2021 guidance emphasis on the problematic under-representation of *adolescents* means RECs have a substantial basis for making inquiries about plans to enrol adolescents. Because of shifts away from vulnerable people to *contexts of vulnerability*, RECs should be alert for descriptions about contexts that may increase vulnerabilities to harms, for example laws that criminalize same-sex behaviour or underage consensual sexual activity among adolescent peers [24]. A shift to highlight gender diversity paves the way for RECs to make insightful inquiries about plans to reach gender-diverse adolescents, and even inquire about prior training on such matters for study staff. Subtle shifts in consent language mean that RECs should be more aware of the potential consequences of parental consent strategies. The acceptability of parental waivers in some contexts depends on whether interventions present an acceptable level of risk [23], and risks of HIV preventive vaccine trials in adolescents (including social impacts from Vaccine Induced Sero-Positivity [25]) will require careful attention by RECs when considering the appropriate consent approach to adolescent enrolment.

2.4 | Enrolment of pregnant women

Some have argued that HIV preventive trials involving pregnant women lag behind HIV treatment trials [26]. However, only one HIV vaccine regimen has shown even modest efficacy [27] that did not justify testing the regimen during pregnancy. This is in contrast to HIV treatment trials where several regimens have demonstrated sufficient safety and efficacy in non-pregnant women to justify enrolment of pregnant women. A recent review encourages guideline-developers (among others) to "affirm the imperative for responsible research with pregnant women" [28].

In a similar vein, UNAIDS 2021 [13] shifts to underscoring problematic evidence gaps and inequities in access for groups, including "pregnant women or gender diverse persons", even while its predecessor opposed exclusion of vulnerable groups like pregnant women without scientific reasons. Like its predecessor, the 2021 document recommends timely discussion to resolve the exclusion of pregnant persons. But the 2021 document shifts the timing of such discussions *earlier* – when the candidate "has sufficient promise to advance into phase 2b or 3" – and removes the choice to time the discussion after the trial product has been demonstrated effective. Also, the 2021 guidance shifts the topic of discussion to "pregnancy-specific pharmacokinetic (PK)" studies away from "safety studies". These shifts resonate with recommendations for the early integration of pregnancy-specific pharmacokinetic studies into development plans so that new interventions "reach the market with pregnancy-specific dosing information" at or closely after licensure [28].

It is also important to explore whether experimental HIV vaccines administered to infants born to women living with HIV (in combination with known safe and effective antiretroviral treatment [ARTs]) can prevent transmission of HIV from the mother. For example, HVTN 135 is enrolling mother–infant pairs in South Africa, and is vaccinating infants. The UNAIDS 2021 guidance document asserts that trials to prevent mother-to-child transmission are outside the scope of the guidance, even while many guidance points remain relevant.

In terms of ethics review of *enrolment of pregnant women and gender diverse persons*, the shift towards an equitable evidence base for pregnant persons in UNAIDS 2021 means that RECs ought to "proactively work with investigators to identify approvable designs" [28]. It follows that RECs should not *generally* default to exclusion but adopt the "burden of careful analysis" of each protocol *individually* [29] to see if acceptable risk–benefit ratios are met. RECs are responsible for assessing whether the risks of study procedures/interventions are justified by either the potential for direct benefit to the pregnant person and/or the foetus, or by generalizable knowledge to other pregnant persons and foetuses and in the latter instance, no greater risk than "minimal harm" to the foetus is required [29]. Assessing if a favourable risk–benefit ratio can be met for each component of the trial in a component analysis can be a useful approach [30], and RECs should carefully document their inclusion/exclusion decisions [29]. In terms of persons who become pregnant while participating in trials, RECs should ensure all protocols address collection of relevant safety data from the pregnant participant, and data on

the outcome of the pregnancy. In some protocols, collection of safety data on the newborn/infant may be justified. Having experts in maternal/foetal medicine on RECs may be an important action in supporting quality ethics review.

3 | CONCLUSIONS

There have been several shifts in the UNAIDS ethics guidance with implications for HIV vaccine researchers submitting applications for initial review or re-certifications, *and* for RECs conducting reviews. These shifts, made in response to contemporary challenges in the field, may not harmonize completely with other leading guidance; for example, UNAIDS 2021 standard of prevention is "WHO-recommended", whereas CIOMS 2016 recommends "established effective" and HPTN 2020 recommends "known effective", "practically achievable", and "reasonably accessible". A thorough head-to-head comparative analysis is needed to identify where substantive differences are large on key concerns, and advocacy will be needed to resolve tensions that threaten the ethics evaluation of HIV vaccine studies. This paper may go some way towards indirectly improving efficiency and consistency in review processes [4,31,32]. However, additional tool development and training may further help affected stakeholders to recalibrate protocol development and ethics review in a way that reflects the impact of new UNAIDS ethics recommendations.

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COMPETING INTERESTS

CS serves on a DSMB that has oversight of certain HIV vaccine trials and DSMB members receive an honorarium from NIAID. She was part of the consultation hosted by WHO in the process of guideline revision.

PN serves on a Research Ethics Committee that has oversight of certain HIV vaccine trials.

MA is employed by the National Institutes of Health, a US government agency that has funded clinical trials of preventive HIV vaccines and monoclonal antibodies, and she has served as medical officer for such trials.

JS has no competing interests.

AUTHORS' CONTRIBUTIONS

CS conceptualized the commentary, reviewed the guidance and developed the first draft. PN, MA and JS revised the article for important content.

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REVIEW

Current approaches to HIV vaccine development: a narrative review

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Abstract

Introduction: The development of an effective vaccine to protect against HIV is a longstanding global health need complicated by challenges inherent to HIV biology and to the execution of vaccine efficacy testing in the context of evolving biomedical prevention interventions. This review describes lessons learnt from previous efficacy trials, highlights unanswered questions, and surveys new approaches in vaccine development addressing these gaps.

Methods: We conducted a targeted peer-reviewed literature search of articles and conference abstracts from 1989 through 2021 for HIV vaccine studies and clinical trials. The US National Library of Medicine's Clinical Trials database was accessed to further identify clinical trials involving HIV vaccines. The content of the review was also informed by the authors' own experience and engagement with collaborators in HIV vaccine research.

Discussion: The HIV vaccine field has successfully developed multiple vaccine platforms through advanced clinical studies; however, the modest efficacy signal of the RV144 Thai trial remains the only demonstration of HIV vaccine protection in humans. Current vaccine strategies include prime-boost strategies to improve elicitation of immune correlates derived from RV144, combination mosaic antigens, novel viral vectors, antigens designed to elicit broadly neutralizing antibody, new nucleic acid platforms and potent adjuvants to enhance immunogenicity across multiple classes of emerging vaccine candidates.

Conclusions: HIV vaccine developers have applied lessons learnt from previous successes and failures to innovative vaccine design approaches. These strategies have yielded novel mosaic antigen constructs now in efficacy testing, produced a diverse pipeline of early-stage immunogens and novel adjuvants, and advanced the field towards a globally effective HIV vaccine.

Keywords: Adjuvant; HIV; vaccines

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1 | INTRODUCTION

Human immunodeficiency virus (HIV) remains a widespread and compelling global health threat with 38 million people living with HIV and 1.7 million new cases in 2019 [1]. Global deployment of antiretroviral therapy and an increasing armamentarium of non-vaccine HIV prevention tools are being employed to combat the epidemic, but, as discussed elsewhere in this special issue, an effective vaccine will likely be needed to end it [2]. Many technologic advances and research capabilities of the HIV vaccine field have been leveraged rapidly to develop highly successful SARS-CoV-2 vaccines; however, among HIV vaccine efficacy trials completed to date, the RV144 Thai trial remains the only study to demonstrate a positive signal with an estimated efficacy of 31% at 3.5 years [3]. We will review current approaches to improve on that result, including vaccine candidates in efficacy trials as well as platforms in earlier stages of clinical development.

2 | METHODS

We conducted a targeted peer-reviewed literature search employing PubMed/MEDLINE to access material from 1989 to 2021 and utilized the keywords HIV and/or vaccine, trimer, broadly neutralizing antibody (bNAb), mRNA, adjuvant. The U.S. National Library of Medicine's Clinical Trials database was searched to further identify HIV vaccine clinical trials. The review was informed by the authors' own experience in the HIV vaccine field and was completed on 18 March 2021.

3 | DISCUSSION

3.1 | Challenges to HIV vaccine development

Development of a globally effective HIV vaccine faces manifold challenges. HIV's characteristic sequence diversity poses a formidable obstacle, with circulating strains differing from

one another by approximately 20% in relatively conserved proteins and up to 35% in the envelope (Env) [4,5]. HIV has multiple transmission routes, including intravenous, vaginal or intrarectal sexual transmission, all of which result in widespread systemic viral dissemination within one to two weeks post exposure [6–10], seeding long-lived cellular populations [11,12] and immune-privileged anatomic reservoir sites [13–16]. HIV envelope glycans, critical for Env folding and cleavage during maturation [17–19], also enable HIV to evade the immune system by hiding epitopes [20]. Furthermore, HIV proteins such as Vif and Vpu antagonize host viral restriction factors [21–25].

In addition to technical obstacles, HIV vaccine efficacy testing is challenged by logistical considerations and advancements in non-vaccine prevention modalities such as preexposure prophylaxis (PrEP), which must be reflected in the design and conduct of efficacy trials. Ethical considerations for incorporating PrEP into preventive HIV vaccine efficacy trials are further discussed in the commentary by Slack, *et al.* in this issue [26].

However, despite the rapidly evolving virus and prevention landscape, the HIV vaccine field has developed multiple candidates through the successful implementation of efficacy trials (Table 1), demonstrated initial efficacy with RV144, and is now advancing a panoply of new vaccine design approaches into the clinic. These accomplishments highlight the feasibility and promise of a licensed HIV vaccine to end the epidemic.

3.2 | Early vaccine efficacy trials

3.2.1 | *VAX003 and VAX004*

The first Phase 3 HIV vaccine efficacy trials were VAX003 and VAX004, which both utilized a bivalent protein formulation with alum adjuvant. VAX003 participants were persons who inject drugs in Thailand and utilized bivalent subtype B and AE proteins [27,28], and VAX004 enrolled men who have sex with men (MSM) and women at risk for heterosexual acquisition of HIV in the Americas with bivalent subtype B proteins [29,30]. In both trials, the vaccines did not prevent HIV infection, and there was no significant effect on viral load, $CD4^+$ T-cell count, or disease progression [31], suggesting that bivalent protein vaccination alone is insufficient to induce protective efficacy.

3.2.2 | *STEP (HVTN 502) and Phambili (HVTN 503) trials*

Results of the VAX003 and VAX004 trials prompted a shift to strategies for eliciting HIV-specific T-cell responses with vector-based vaccines. The STEP trial evaluating a replication defective adenovirus serotype 5 (Ad5) vectored vaccine enrolled MSM, sex workers, and participants with elevated heterosexual risk in the Americas and Australia [32]. The trial was halted by the Data Safety Monitoring Board (DSMB) after initial data showed an increase in HIV infection among uncircumcised males and/or Ad5 seropositive vaccinees. Interim analysis revealed an infection rate in per protocol male vaccinees double that of placebo recipients. The Phambili trial, which enrolled participants in the Republic of South Africa (RSA) [33] was terminated early based on the results of the STEP trial, and preliminary data revealed a higher incidence of HIV infection in the vaccinated group compared to placebo, without impact on viral load or disease progression. *In vitro* experiments demonstrated that Ad5-specific $CD4^+$ T cells are highly susceptible to HIV infection [34], and that these cells are preferentially lost in HIV-1-positive individuals [35]. These studies raised important questions about pre-existing anti-vector immunity and concern about the use of Ad5 vectored vaccines where Ad5 is prevalent.

3.2.3 | *RV144 Thai trial*

RV144, performed by the United States Army and the Thai Ministry of Public Health with support from the National Institutes of Health (NIH), was conducted from 2003 to 2009 in Rayong and Chon Buri provinces and enrolled over 16,000 participants at relatively low heterosexual risk for HIV infection. This was the first efficacy trial employing a pox-protein prime-boost strategy: ALVAC-HIV (vCP1521), a recombinant canarypox vector, was boosted with the bivalent protein AIDSVAX B/E adsorbed to aluminium hydroxide aiming to elicit both cellular and humoral responses. The regimen showed 60% efficacy at 12 months post immunization; however, this waned to 31% at 3.5 years [36]. Despite the modest result, RV144 rekindled hope in the feasibility of an effective HIV vaccine and raised the central question of improving durability of protection.

Extensive analyses enabled identification of immune correlates of risk from RV144 (Table 2) [37–45]. Importantly, the vaccine did not elicit neutralizing antibodies, opening the door to investigate non-neutralizing mechanisms of protection. IgG antibody binding to the V1V2 region of envelope correlated inversely with the rate of HIV acquisition, and binding of plasma IgA antibodies to envelope correlated directly with the rate of infection. The correlates analysis also indicated that avidity of IgG for envelope, antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis (ADCP), and Env-specific $CD4^+$ T cells were inversely correlated with risk of infection. Subsequent sieve analysis revealed that the vaccine caused selective effects on the V2 region in breakthrough viruses [45].

3.2.4 | *HVTN 505 trial*

The next efficacy results reported were those of HVTN 505, which was designed to answer questions about Ad5 vectors left outstanding by the STEP/Phambili results. It was conducted in the United States in Ad5 sero-negative men and transgender females who have sex with men to test a DNA/rAd5 prime-boost regimen, inclusive of Env, Gag, Pol and Nef immunogens [46]. Of note, the V1V2 regions were deleted from the subtype B Env gene in this formulation. The DSMB halted the trial after the first interim analysis revealed that the vaccine did not prevent infection nor reduce HIV viral load [47]. *Post hoc* analysis of HVTN 505 revealed that ADCP, binding to FcγRIIa, and HIV-1 Env IgG3 correlated to reduced acquisition risk of HIV [48]. Additional work revealed that certain polymorphisms of FcγR modulate HIV acquisition after

Table 1. Summary of HIV vaccine efficacy trials

Study	Immunogen	Adjuvant	Schedule	Location/study population	Date	Efficacy	Reference
VAX003 (NCT00006327)	AIDSVAX B/E (subtype B - MN; subtype AE - A244 rgp120)	Aluminium hydroxide	M 0/1/6/12/18/24/30	Thailand/persons who inject drugs	March 1999 to August 2000	No	[27,28,31]
VAX004 (NCT00002441)	AIDSVAX B/B (subtype B - MN and GNE8 rgp120)	Aluminium hydroxide	M 0/1/6/12/18/24/30	North America, Nether-lands/MSM and high-isk women	2001 to 2003	No	[29–31]
STEP HVTN 502	Ad5 expressing subtype B Gag (CAM-1), Pol (IIIB), Nef (JR-FL)		M 0/1/2/6	Americas, Australia/MSM and high-risk heterosexual men and women	December 2004 to March 2007	No	[32,34,35]
Phambili HVTN 503 (NCT00413725)	Ad5 expressing subtype B Gag (CAM-1), Pol (IIIB), Nef (JR-FL)		M 0/1/2/6	Republic of South Africa/ heterosexual men and women	January 2007 to September 2007	No	[33]
RV144 (NCT00223080)	ALVAC-HIV (vCP1521) expressing Gag and Pro (subtype B LAI), CRF01_AE gp120 (92TH023) linked to transmembrane anchoring portion of gp41 (LAI) AIDSVAX B/E	Aluminium hydroxide	M 0/1/3/6	Thailand/relatively low-risk men and women	October 2003 to July 2009	Yes - 31%	[36–45]
HVTN 505 (NCT00865566)	6 DNA plasmids - subtype B Gag, Pol, Nef and subtypes A, B and C Env 4 rAd5 vectors - subtype B Gag/Pol and subtypes A, B and C Env		D 0/28/56/168	USA/Ad5 seronegative MSM	May 2009 to October 2017	No	[46–49]
HVTN 702 (NCT02968849)	ALVAC-HIV (vCP2438) expressing Gag and Pro (subtype B LAI), subtype C gp120 (ZM96.C) linked to transmembrane anchoring portion of gp41 (LAI) Bivalent C gp120 (TV1 C/1086 C)	MF59	M 0/1/3/6/12 (18)	Republic of South Africa/ heterosexual men and women	October 2016 to (Proj) September 2021	No	
IMBOKODO HVTN 705 (NCT03060629)	Ad26.Mos4.HIV Subtype C gp140	Aluminium phosphate	M 0/3/6/12	RSA, Malawi, Mozambique, Zambia, Zimbabwe/ women	November 2017 to (Proj) July 2022		

(*Continued*)

Table 1. Continued

Study	Immunogen	Adjuvant	Schedule	Location/study population	Date	Efficacy	Reference
MOSAICO HVTN 706 (NCT03964415)	Ad26.Mos4.HIV Subtype C gp140 or bivalent gp140 (subtype C/Mosaic)	Aluminium phosphate	M 0/3/6/12	US, Latin America, Italy, Spain/cis-gender men and transgender individuals who have sex with cis-gender men and/or transgender Individuals	October 2019 to (Proj) January 2023		
PrEPVacc (NCT04066881)	DNA-HIV-PT123 plasmid and AIDSVAX B/E or DNA-HIV-PT123 plasmid with trimeric CN54gp140; MVA-CMDR (Chang Mai double recombinant) and trimeric CN54gp140 Concurrent PrEP administration of either TAF/FTC or TDF/FTC	Aluminium hydroxide or MPLA	M 0/1/6/12	Uganda, Tanzania, Mozambique, Republic of South Africa/men and women	(Proj) January 2020 to (Proj) March 2023		
AMP Study HVTN 703/HPTN 081 (NCT02568215) HVTN 704/HPTN 085 (NCT02716675)	Monoclonal antibody VRC01		M 0/2/4/6/8/10/12/14/16/18	(HVTN 703) Botswana, Kenya, Malawi, Mozambique, Republic of South Africa, Tanzania, Zimbabwe/women (HVTN 704) US, Brazil, Peru, Switzerland/men	(HVTN 703) April 2016 to December 2020 (HVTN 704) May 2016 to March 2021	No	[76]

Abbreviations: Proj, projected; MSM; men who have sex with men; RSA, Republic of South Africa; US, United States.

vaccination, demonstrating the potential for host genetics to impact vaccine efficacy [49].

3.3 | Follow-up trials building on RV144

3.3.1 | RV305, RV306 and RV328 trials

With the observation of waning immunity and time-dependent efficacy in RV144 [3,36], two early-phase trials examined strategies to improve durability of responses by boosting with vaccine regimen components. RV305 enrolled vaccinated individuals without HIV from RV144 after six to eight years, evaluating two immunizations of ALVAC-HIV, AIDSVAX B/E, or both compared to placebo. ALVAC-HIV boosting alone did not induce significant titers against any of the HIV antigens examined. Those who received ALVAC-HIV plus AIDSVAX B/E or AIDSVAX B/E alone had IgG responses against gp120 and gp70-V1V2 at levels significantly higher than peak immunogenicity in RV144, indicating that the priming RV144 vaccination series six to eight years earlier had evoked memory responses [50]. Interestingly, the magnitude of those increases was higher after the initial late boost at month 0 than after the second late boost at month 6, suggesting that the duration of the boosting interval influences responses. Repeated boosting induced antibodies with qualities of

Table 2. Correlates of HIV protection determined in RV144

		Reference
Antibody binding to Env	IgG to V2	[37,38]
	IgG to V2 and V3	[39]
	IgG3 to V1V2	[40]
	Avidity of IgG for Env	[37]
	Low IgA	[41]
Antibody functions	IgG mediated ADCC	[42]
	IgG3 associated ADCC and ADCP	[43]
	Lower IgA enables ADCC function	[41]
Cellular	Polyfunctional Env-specific $CD4^+$ T cells	[44]

Table 3. Summary of RV144 and HVTN 702 efficacy trials

		RV144	HVTN 702
Viral	Viral subtype	AE	C
	Viral diversity	Relatively homogenous	Highly diverse
Population	HIV risk/incidence	0.28%	Approximately 4%
	Host genetics	Thai	African
Vaccine	Adjuvant	Aluminium hydroxide	MF59
	ALVAC inserts	Subtype AE vCP1521	Subtype C vCP2438
	Protein boost	Bivalent AE/B (A244/MN)	Bivalent C (TV1 C/1086 C)
	Protein dose	Higher (300 μg of each protein)	Lower (100 μg of each protein)
	Dosing schedule	M 0/1/3/6	M 0/1/3/6/12 (18)

bNAbs such as greater somatic hypermutation and longer immunoglobulin heavy-chain complementarity-determining region 3 (HCDR3) length [51]. However, an additional delayed boost skewed responses towards the IgG4 subclass, associated with reduced non-neutralizing function (unpublished data). Therefore, boosting repeatedly with the same antigen may fall short in achieving durably functional immune responses. This trial also confirmed that similar HIV-specific antibody responses were present in cervicovaginal and rectal secretions and semen, which, in RV144, may have impacted early viral pathogenesis, resulting in prevention of infection or interruption of critical steps early in acquisition [52].

RV306 recapitulated the RV144 regimen and added boost immunizations with either AIDSVAX B/E (12 months) or ALVAC-HIV/AIDSVAX B/E (12, 15 or 18 months) to determine optimal boost timing. The impact of late boosting was demonstrated by lower plasma IgG responses at 24 months in those receiving the boost at 12 months compared to those receiving the immunizations at 15 and 18 months. Delayed boosting also increased $CD4^+$ T-cell functionality and polyfunctionality [53].

3.3.2 | *HVTN 097 and HVTN 100 trials*

Following the promising results from RV144, HVTN 097 was conducted in RSA employing the RV144 vaccine formulation and schedule. The study demonstrated overall response rates of plasma IgG and Env-specific $CD4^+$ T cells expressing IFN-γ and/or IL-2 similar to those elicited in RV144 [54,55].

HVTN 100 was conducted in RSA with a pox-protein regimen re-tooled for the South African subtype C epidemic: the ALVAC-HIV vCP2438 expressed HIV subtype C gp120, subtype B gp41, gag and protease followed by a bivalent subtype C (TV1/1086) gp120 boost. Also, an alternative adjuvant, the MF59 oil-in-water emulsion, was substituted for the aluminium hydroxide used in RV144. This vaccine regimen had a similar schedule to RV144, with an additional boost at month 12, and was shown to be safe and well tolerated. All vaccine recipients developed gp120 binding antibodies, which were significantly increased compared to RV144. The regimen also induced higher $CD4^+$ T-cell responses to the corresponding envelope protein. Although IgG antibody responses directed at 1086_V1V2 were lower in HVTN 100 relative to those elicited by the RV144 regimen in HVTN 097 (70.5% vs. 99%), responses exceeded the predicted threshold needed for 50% vaccine efficacy, and the results from this trial met the go-no-go criteria for advancing to the HVTN 702 trial [56].

3.3.3 | *HVTN 702 Uhambo efficacy trial*

Uhambo, the HVTN 702 Phase 2b/3 study in RSA, began in 2016 and enrolled participants at risk for HIV. These participants were sexually active men and women aged 18 to 35 and were offered the local HIV-prevention standard of care, including access to PrEP. In February 2020, interim analysis results revealed that there was no significant evidence of decreased or increased infection rates associated with the vaccine regimen. The analysis was performed with 2694 vaccinees and 2689 placebo recipients, with 129 HIV infections among the vaccinees and 123 HIV infections among the placebo recipients. Given this lack of efficacy, The National Institute of Allergy and Infectious Diseases (NIAID) halted the trial at the recommendation of the DSMB.

HVTN 702 was highly successful operationally; however, the results indicating the vaccine failed to reproduce or amplify the efficacy of RV144 were disappointing. To understand this outcome, it is useful to examine the differences between the two studies (Table 3).

First, the targeted viruses and populations were both very different. RV144 was conducted in a CRF01_AE predominant region, and HVTN 702 was conducted where subtype C virus circulates. Also, the population studied in Thailand was at dramatically lower risk for infection, as the HIV incidence was almost 15-fold lower in Thailand (0.28%) compared to RSA (approximately 4%). Differences in host genetics of the populations participating in the clinical trials may have played a role [57]. Critically, the vaccine components also differed substantially between the regimens: both the ALVAC inserts and the protein boosts differed. The amount of bivalent protein administered also varied, with a dose of 600 μg in RV144 and

200 μg in HVTN 702. The lower dose of the bivalent gp120 was administered due to the use of MF59, which contrasted with the aluminium hydroxide used in RV144.

In considering the discrepant outcomes of these studies, the critical question to be answered is - what provides protection? For example, the implications of using a different adjuvant may be significant: a non-human primate (NHP) study examined MF59 versus aluminium hydroxide. The study standardized the baseline risk of infection, challenge virus and other vaccine components. Protection was observed only among animals receiving aluminium hydroxide – containing immunizations. Although higher plasma antibody titers were observed with MF59, they did not protect from acquisition, and mucosal antibodies were the outcome that correlated with protection [58]. This study underscores that higher magnitude of immune responses does not necessarily correspond to better protection and that the quality and location of the response is important. Also, genes expressed after vaccination may play a role in protection. The RV144 vaccine regimen strongly induced the IFNγ pathway, more specifically the activation of IRF7, which was associated with reduced risk of acquisition of infection [59].

Although HVTN 702 was unsuccessful protecting against HIV acquisition, investigators can further examine factors that may have impacted infection within the study population. To inform subsequent progress in HIV vaccine development, it is critical to understand correlates of risk from HVTN 702 across immune and anatomic compartments. These findings can then provide insight for approaching the current diversity of strategies being undertaken to achieve a successful HIV vaccine (Table 4).

3.3.4 | *HVTN 111 trial*

While HVTN 100 (and subsequently HVTN 702) adopted a pox-protein approach to parallel RV144, HVTN 111 adopted a DNA priming approach, employing a subtype C DNA prime (DNA-HIV-PT123) with the same bivalent subtype C gp120 boost used in the HVTN 100 regimen. Evaluating strategies for enhancing RV144 correlates of protection, Moodie *et al*. examined antibody and cellular responses of both HVTN 100 and HVTN 111 [60]. Although both the ALVAC and DNA priming regimens induced high binding and neutralizing titers, the DNA prime induced significantly higher $CD4^+$ T-cell response rates compared to the ALVAC prime. Interestingly, the vaccinees from HVTN 111 had higher magnitude of binding antibodies to the V1V2 region and had higher neutralizing responses to tier-1 viruses compared to the HVTN 100 vaccinees. This study highlighted the potential for DNA priming to enhance the magnitude of elicited immune responses in protein-boosted HIV vaccine regimens.

3.4 | New approaches for an HIV vaccine

3.4.1 | *Ad26 mosaic trials*

The Ad26 mosaic vaccine program developed by Janssen Pharmaceuticals has combined viral vectors (adenovirus serotype 26 or modified vaccinia Ankara - MVA), protein boosts and immunogen sequences optimized to address global HIV diversity in the creation of polyvalent "mosaic" antigens. Fischer *et al*. explained the approach for designing antigens that focus on eliciting T-lymphocyte responses as well as neutralizing antibodies by including Env as part of the design [61]. The mosaic antigens are generated from natural sequences, which include common potential epitopes, while excluding rare sequences.

The clinical use of these mosaic antigens was informed by a series of NHP studies [62–64], and the initial regimen selection clinical trial for this platform was the APPROACH study. This Phase 1/2 trial assessed seven regimens containing Ad26 or MVA vectors expressing mosaic antigens, with some groups boosted with high or low doses of gp140 adsorbed to aluminium phosphate. All regimens were safe and well tolerated [65], and binding antibody responses to autologous clade C gp140 was detected in all groups following the second prime immunization at month 3. After the first boost immunization at month 6, most groups maintained 100% antibody response. The groups that received the gp140 boost had the highest ELISA antibody titers, the magnitude of which was dose-dependent. In comparison to the gp140-only group, the inclusion of either Ad26.Mos.HIV or MVA-mosaic increased responses. The utility of the mosaic antigens was demonstrated by the ability to elicit binding IgG responses to cross-clade transmitted/founder Envs, Envs isolated from chronically infected individuals, and to consensus sequence Envs that were similar to the vaccine homologous responses [65]. Similar to RV144 vaccinees, a substantial number of subjects showed responses to gp70-V1V2 antigens and the IgG subclasses elicited were IgG1 and IgG3. The highest ADCP responses were observed in the gp140-boosted groups, particularly those primed with a viral vector. Serum neutralizing activity was only detected against tier-1HIV variants [65].

In parallel with the APPROACH study, an NHP study (13-19) was conducted and observed similar immunogenicity. All animals underwent six intrarectal challenges with SHIV-SF162P3, with the Ad26.Mos.HIV/gp140 (aluminium phosphate) group demonstrating the most favourable results: eight out of twelve animals were protected from the entire series of challenges, demonstrating a 94% reduction in per acquisition risk and 66% complete protection [65].

Four more clinical trials have built on these initial findings: TRAVERSE, IMBOKODO, ASCENT, and MOSAICO. TRAVERSE is a Phase 1/2a study that assessed the safety and immunogenicity of a trivalent Ad26.Mos.HIV compared to a tetravalent Ad26.Mos4.HIV prime in the context of a subtype C gp140 boost adjuvanted with aluminium phosphate. All vaccinees developed gp140 binding IgG antibodies after two immunizations of either the trivalent or tetravalent formulations; however, the tetravalent formulation elicited higher total IgG geometric mean titers. ADCP function and Env-specific $CD4^+$ T-cell response rates were also improved with the tetravalent vaccine [65], and the Ad26.Mos4.HIV formulation has advanced into the IMBOKODO proof-of-concept efficacy trial. This Phase 2b study conducted in southern Africa has completed enrollment with women at risk for HIV, and the primary completion date is projected for 2022.

ASCENT is a Phase 1/2a study that assessed the Ad26.Mos4.HIV prime with either a monovalent subtype C gp140 boost or a bivalent combination of subtype C gp140 and mosaic gp140. Both regimen boosts were adjuvanted

Table 4. Summary of early-phase HIV vaccine trials

Study	Phase	Immunogen	Adjuvant	Schedule	Location/study population	Date	Reference
HVTN 097 (NCT02109354)	1	ALVAC-HIV (vCP1521) expressing Gag and Pro (subtype B LAI), CRF01_AE gp120 (92TH023) linked to transmembrane anchoring portion of gp41 (LAI) AIDSVAX B/E	Aluminium hydroxide	M 0/1/3/6	RSA/ heterosexual men and women	June 2013 to February 2015	[54,55]
HVTN 100 (NCT02404311)	1, 2	ALVAC-HIV (vCP2438) expressing Gag and Pro (subtype B LAI), subtype C gp120 (ZM96.C) linked to transmembrane anchoring portion of gp41 (LAI) Bivalent C gp120 (TV1 C/1086 C)	MF59	M 0/1/3/6/12 (18)	RSA/ heterosexual men and women	February 2015 to August 2018	[56]
HVTN 111 (NCT02997969)	1	DNA-HIV-PT123: DNA plasmid encoding subtype C Gag (ZM96), Env (ZM96), and Pol-Nef (CN54) Bivalent C gp120 (TV1/1086)	MF59	M 0/1/3/6	RSA, Tanzania, Zambia/men and women	June 2016 to July 2018	[60]
APPROACH HIV-V-A-004 IPCAVD 009 (NCT02315703)	1, 2	Ad26.Mos.HIV vector followed by boosts of Ad26.Mos.HIV and gp140 (high or low dose); Ad26.Mos.HIV alone; MVA-mosaic and gp140 (high or low dose); MVA-mosaic alone; or gp140	Aluminium phosphate	M 0/1/3/6/12	USA, Rwanda, RSA, Thailand, Uganda/men and women	December 2014 to (Proj) July 2022	[65]
TRAVERSE HVTN 117 (NCT02788045)	1, 2a	Ad26.Mos.HIV or Ad26Mos4.HIV Subtype C gp140	Aluminium phosphate	M 0/3/6/12	USA, Rwanda/men and women	July 2016 to (Proj) April 2023	[66]
ASCENT HVTN 118 (NCT02935686)	1, 2a	Ad26.Mos4.HIV Subtype C gp140 or bivalent gp140 (subtype C/mosaic)	Aluminium phosphate	M 0/3/6/12	USA, Kenya, Rwanda/men and women	March 2017 to (Proj) May 2023	[67]
HIVCORE 005/6 (NCT04553016)	1	ChAdOx1.tHIVconsv1 MVA.tHIVconsv3 and MVA.tHIVconsv4		M 0/1	Kenya, Uganda, Zambia/men and women	(Proj) March 2021 to (Proj) June 2022	
IAVI W001 (NCT03699241)	1	BG505 SOSIP.664 gp140	AS01B		USA, Kenya/men and women	December 2018 to (Proj) May 2020	
ACTHIVE-001 (NCT03961438)	1	ConM SOSIP.v7 gp140	MPLA	M 0/2/6	Netherlands/men and women	November 2019 to (Proj) November 2022	
EAVI2020_01 (NCT03816137)	1	ConM SOSIP, EDC ConM SOSIP, ConS UFO, EDC ConS UFO and mosaic SOSIPs	MPLA	M 0/3/6/12	UK/men and women	March 2019 to (Proj) December 2021	
IAVI G001 (NCT03547245)	1	eOD-GT8 (self-assembling nanoparticle with HIV Env)	AS01B		USA/men and women	June 2018 to (Proj) December 2020	

(*Continued*)

Table 4. Continued

Study	Phase	Immunogen	Adjuvant	Schedule	Location/study population	Date	Reference
IAVI C101 (NCT04224701)	1	BG505 SOSIP GT1.1 gp140	Present (from GSK)	M 0/2/6	USA, Netherlands/men and women	August 2020 to (Proj) July 2022	
IAVI study (NCT01264445)	1	Ad35 expressing Gag, RT, Int, and Nef; fusion protein expressing subtype B p24, RT, Nef and p17	AS01	M 0/1/4	Kenya, Uganda, Zambia/men and women	February 2011 to November 2012	[10]
RV546 (NCT04658667)	1	Full-length single-chain (FLSC) gp120-CD4 chimera subunit HIV-1 vaccine (IHV01), A244 gp120	Aluminium hydroxide ALFQ	D 0	Thailand/RV306 participants	(Proj) August 2021 to (Proj) October 2022	
HVTN 137 (NCT04177355)	1	BG505 SOSIP.664 gp140	3M-052-AF CpG 1018 GLA-LSQ Aluminium hydroxide	M 0/2/(6)	USA/men and women	January 2020 to (Proj) May 2022	

Abbreviations: Proj, projected; RSA, Republic of South Africa; USA, United States of America.

with aluminium phosphate and showed high Env-specific binding antibody levels with similar subclass distribution. Clade C responses were not attenuated by the half dose administered in the group containing the mosaic gp140 boost, which also demonstrated improved subtype B responses. $CD4^+$, but not $CD8^+$, T-cell responses were increased in the bivalent regimen compared to the subtype C gp140 alone [67]. This Ad26 mosaic vaccine regimen with bivalent protein boosting has subsequently advanced into the MOSAICO Phase 3 licensure trial in the Americas and Europe, which began in October 2019 and plans an estimated enrollment of 3800 cis-gender men and transgender individuals who have sex with cis-gender men and/or transgender individuals. If efficacy is seen in IMBOKODO and/or MOSAICO, those results will add to the modest success of RV144 to demonstrate the ability of non-neutralizing antibody and cellular mechanisms to afford protection and expand our knowledge of correlates of protection to facilitate bridging studies to additional populations and geographic regions.

Geographic and participant diversity as well as broad collaboration have been strengths of the Janssen clinical programme, which has engaged multiple HIV vaccine research institutions, networks and laboratories since its inception. The mosaic concept is being put to test with efficacy efforts across multiple HIV subtypes as well as a diversity of participant gender, demographic and risk factors with appropriate engagement of these communities, leveraging established capabilities of the HVTN and other partners. All these considerations have implications not just for demonstrating and understanding vaccine protection, but also for the eventual deployment of vaccines to communities at risk.

3.4.2 | *PrEPVacc trial*

Another highly collaborative effort, PrEPVacc, is an African-led vaccine efficacy trial supported by the European and Developing Countries Clinical Trials Partnership (EDCTP) incorporating a PrEP comparison into a study design evaluating two vaccine candidates. The first vaccine regimen, previously evaluated in HVTN105 (NCT02207920), is a DNA-HIV-PT123 plasmid prime (as in HVTN111) plus an AIDSVAX B/E boost. The second vaccine regimen is a DNA-HIV-PT123 with CN54gp140 prime followed by an MVA-CMDR (Chang Mai double recombinant) and CN54gp140 boost. The two vaccine regimens will be compared against placebo, and participants are offered PrEP with TDF/FTC or TAF/FTC during the period of the first three immunizations. PrEPVacc utilizes a multiarm, multistage adaptive trial design that enables the efficient evaluation of multiple vaccine regimens and also employs an averted infections ratio methodology to determine efficacy. Vaccinations have commenced and the study is estimated to be completed in 2023.

3.4.3 | *New viral vectors*

Given the insufficient efficacy seen with Ad5 and ALVAC vaccines, other viral vectors are being explored for improved HIV vaccine regimens. Strategies employing Ad26 or Ad35 vectors have been shown to elicit robust humoral and cellular immune responses, and importantly, pre-existing anti-vector antibodies do not affect vaccine safety or immunogenicity [68–71]. Ad4 is a live virus vaccine vector under clinical evaluation. NCT01989533 is examining an Ad4-HIV vaccine regimen consisting of an Ad4-mosaic Gag prime and an Ad4-EnvC150 boost. The vaccine is formulated as enteric-coated capsules to be delivered orally or as an aqueous formulation for intranasal administration.

The human cytomegalovirus (CMV) vector is another promising novel vectored approach for HIV vaccines. NHPs vaccinated with CMV/SIV vectors induced persistent and high-frequency SIV-specific memory T-cell responses at potential sites of SIV replication. These responses were

associated with durable aviremic control of SIV infection in 50% of the NHPs, and this protection correlated with $CD8^+$ T-cell responses [72,73]. The first clinical trial to examine the safety and immunogenicity of a CMV vector-based vaccine, VIR-1111, is currently recruiting healthy CMV seropositive participants.

HIVCORE 005/6 is a Phase 1 EDCTP clinical trial testing three experimental vaccines with other viral vectors: mosaic immunogens delivered by a prime-boost regimen containing a chimpanzee adenovirus vector (ChAdOx1.tHIVconsv1) and a dual boost of a nonreplicating MVA vector (MVA.tHIVconsv3 and MVA.tHIVconsv4). The ChAdOx1 vector is a component of the Astra Zeneca COVID-19 vaccine, which has been granted a conditional marketing authorization or emergency use in over 50 countries [74,75].

3.4.4 | *Antibody mediated protection (AMP) trials*

The antibody mediated protection (AMP) trials evaluated the protective efficacy of passive immunization with either low (10 mg/kg) or high (30 mg/kg) doses of the bNAb VRC01 delivered every eight weeks in participants at increased risk for HIV acquisition in sub-Saharan Africa (HVTN 703/HPTN 081) and the Americas (HVTN 704/HPTN 081) [76]. The studies did not demonstrate overall efficacy; however, as pioneered in earlier vaccine efficacy trials, evaluation of breakthrough viruses yielded critical insight. The bNAb was 75% effective against VRC01-sensitive isolates (those with 80% inhibitory concentration less than 1 μg/ml) over the course of the study, demonstrating proof of concept for bNAb prophylaxis. However, only a minority of viruses (30% in the placebo groups) were VRC01-sensitive, highlighting the need for broader and more potent bNAbs, likely in combination targeting multiple HIV epitopes. The results also have implications for active vaccination strategies to elicit bNAb: that bar is high, and to achieve protection through purely neutralizing mechanisms, active vaccines will likely need to target multiple HIV epitopes as well.

3.4.5 | *Native-like envelope trimers*

To actively induce bNAb responses, several groups have been advancing novel envelope immunogens. Native-like envelope trimers are designed to present multiple bNAb epitopes mimicking how these epitopes appear on the Env spike, with the BG505 SOSIP.664 construct emerging as the prototypic native-like soluble trimer [77]. Compared to earlier HIV protein immunogens, these trimers are purer and more thermally stable. The approach to the design of these HIV immunogens is further detailed by Sanders and Derking [78].

Several clinical trials are underway evaluating SOSIP trimers. W001 is a Phase 1 IAVI clinical trial testing the BG505 SOSIP.664 gp140 at various doses in combination with the AS01B adjuvant. The Phase 1 ACTHIVE-001 trial is examining the ConM SOSIP.v7 gp140 adjuvanted with monophosphoryl lipid A (MPLA) liposomes (ConM is a consensus of the consensus sequences of each clade in group M). Lastly, the EAVI2020_01 Phase 1 trial is evaluating prime-boost combinations with various forms of SOSIP gp140s including the ConM SOSIP, EDC ConM SOSIP, ConS UFO, EDC ConS UFO, and mosaic SOSIPs.

3.4.6 | *Serial bNAb germline targeting*

Designing immunogens to engage bNAb precursors and their intermediates through sequential immunization [79,80] is an alternate approach to bNAb elicitation, as further described by Williams *et al.* [81]. At HIVR4P 2021, encouraging results of the IAVI Phase 1 G001 trial were announced. This study demonstrated that the eOD-GT8 60mer, a self-assembling nanoparticle comprised of HIV Env, adjuvanted with AS01B, elicited detectable VRC01-class IgG B cells in 97% of vaccinees showing initial proof of concept in humans for the germline targeting approach. Another vaccine candidate designed to induce bNAbs, adjuvanted BG505 SOSIP.GT1.1 gp140, is being examined at two different doses in the IAVI C101 Phase 1 clinical trial.

3.4.7 | *Messenger RNA vaccines*

With many of its roots in vaccine development for cancer, HIV and other infectious diseases, messenger RNA (mRNA) is gaining momentum as a platform following the striking success of SARS-CoV-2 mRNA vaccines. mRNA uses host cells to manufacture protein immunogens that can elicit potent antibody and cellular responses [82]. The platform has the potential for cost-effective and scalable GMP manufacturing, without concern for genome integration or the formation of infectious particles [83,84].

As of July 2021, two mRNA vaccines have shown over 90% efficacy and received Emergency Use Authorization for the prevention of COVID-19 [85,86]. The success of these products leveraged lessons in mRNA delivery and immunogen stabilization that have been learnt in the HIV context [82,87,88] and are now being applied by multiple groups in the development of new HIV vaccines. For example, a m1ψ-mRNA-LNP construct encoding a variant of clade B HIV R3A Env elicited potent immune responses in mice, and a single 50 μg immunization of mRNA-LNP in NHPs resulted in a significant increase in Tfh cell frequency in the draining lymph nodes compared to those that received double-stranded RNA-adjuvanted Env protein immunization. The mRNA-LNP immunization elicited potent antibody neutralization activity against a highly sensitive clade C virus. These neutralization antibody titers were stable through 12 months post vaccination [89].

Moyo *et al.* explored the potential of a self-amplifying mRNA (saRNA) encoding six highly conserved regions of the HIV genome (Gag, Pol). A single immunization with this RNA formulation induced specific $CD4^+$ and $CD8^+$ T cells in BALB/c mice, which were well maintained 22 weeks after vaccination [90]. The immunization also induced $CD4^+$ and $CD8^+$ memory subtypes. These early results demonstrate promise for the elicitation of durable immune responses with mRNA-based vaccine formulations against HIV.

3.4.8 | *New adjuvants*

The decrease in efficacy from 60% at 12 months post immunization to 31% at 3.5 years in RV144 and rapid decline

in humoral immune responses to many gp120 antigens, highlights the requirement for, increasing durability as well as magnitude of protection [3,36]. New adjuvants have the potential to augment protein vaccine immune responses to achieve this goal.

Four types of adjuvants are currently included in licensed vaccine formulations: aluminium salts [91], liposomes or virosomes [92], oil-in-water emulsions [93] and aluminium salt containing adsorbed MPLA [94]. The AS01 adjuvant system, which includes liposomes containing MPLA and a saponin from the *Quillaja saponaria* tree bark, QS21 [95,96], has been utilized in vaccines for multiple pathogens [97–99], and the AS01B formulation is employed in the highly effective Shingrix herpes zoster vaccine [98]. In a human clinical trial study examining an HIV vaccine regimen containing protein immunizations of gp120/Nef Tat with AS01B, the formulation induced strong and durable $CD4^+$ and $CD8^+$ T-cell responses with multifunctional profiles [100]. More recently, an IAVI clinical trial employed AS01B in an HIV prime-boost vaccine strategy evaluating an Ad35 vectored vaccine (expressing Gag, reverse transcriptase, integrase and Nef) with a fusion protein (expressing clade B p24, RT, Nef and p17) adjuvanted with AS01B. This regimen was well tolerated with durable $CD4^+$ and $CD8^+$ responses [101].

The Walter Reed Army Institute of Research has developed the Army Liposome Formulation (ALF) family of liposomal adjuvants containing MPLA. These formulations can contain aluminium hydroxide gel (ALFA), QS21 (ALFQ) or both (ALFQA). ALF adjuvants are nontoxic and have been employed in vaccine formulations against several diseases including HIV, malaria, and campylobacteriosis [102–106]. Supported by favourable NHP immunogenicity [107], a Phase 1 clinical trial (NCT04268420) of the FMP013 circumsporozoite protein malaria vaccine with ALFQ has begun and safely dose-escalated [108].

In the HIV space, a regimen of live-attenuated MVA encoding HIV-1 Gag, Pol and Env, with a multimeric gp145 protein boost adjuvanted with ALFA resulted in NHP protection from multiple challenges of SHIV-1157ipd3N4 [109]. The RV546 Phase 1 trial will assess a full-length single chain gp120-CD4 chimera subunit HIV-1 vaccine with alum (IHV01) paired with A244 gp120, in the absence or presence of ALFQ. This study will enroll RV306 participants, harnessing extant memory responses and evaluating antibody response durability and breadth of antibodies directed to the CD4-induced (CD4-i) epitope that preferentially binds to the CD4-gp120 complex [110].

The HVTN 137 Phase 1 trial is currently underway evaluating the safety and immunogenicity of BG505 SOSIP.664 with several adjuvants and the imidazoquinolinone TLR-7/8 agonist 3M-052. Part A of the trial dose escalates 3M-052 and alum with gp140, and Part B will utilize the highest tolerated dose of 3M-052 with alum. Part B will also examine other adjuvants with gp140 including CpG with alum, GLA-LSQ (GLA and QS-21) and alum alone. Lessons from exploration of novel adjuvants may yield cross-cutting progress across immunogen platforms targeting both neutralizing and non-neutralizing antibody mechanisms of protection.

4 | CONCLUSIONS

Although effective HIV treatment and prevention options are increasingly available, due to limited accessibility and/or other treatment challenges, the urgent need for an HIV vaccine remains. Competing strategies emphasize particular immunologic approaches; however, the exceptional diversity and resilience of HIV will likely necessitate a multiplicity of reinforcing immunologic mechanisms for success. As vaccine technologies and clinical trial networks developed to combat HIV were leveraged in the SARS-CoV-2 response, the unprecedented collaboration, transparency, industry engagement and rapid clinical translation with progression to parallel efficacy testing for multiple vaccine concepts that characterized the coronavirus response can propel HIV vaccine development. Important efficacy trials are underway and innovative HIV vaccine design approaches have yielded a robust and diverse pipeline of early-stage candidates with the potential to incorporate next-generation adjuvants. The stage is set for HIV vaccine developers to move these concepts forward together to achieve the ultimate public health tool of an effective HIV vaccine.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

JK drafted the initial version of the manuscript. SV, JHK and JAA reviewed and revised the manuscript. All authors approved the final version of the manuscript.

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DISCLAIMER

The views expressed are those of the authors and should not be construed to represent the positions of the U.S. Army, the Department of Defense, or HJF.

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REVIEW

Structure-guided envelope trimer design in HIV-1 vaccine development: a narrative review

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Abstract

Introduction: The development of a human immunodeficiency virus 1 (HIV-1) vaccine remains a formidable challenge. An effective vaccine likely requires the induction of broadly neutralizing antibodies (bNAbs), which likely involves the use of native-like HIV-1 envelope (Env) trimers at some or all stages of vaccination. Development of such trimers has been very difficult, but much progress has been made in the past decade, starting with the BG505 SOSIP trimer, elucidation of its atomic structure and implementing subsequent design iterations. This progress facilitated understanding the weaknesses of the Env trimer, fuelled structure-guided HIV-1 vaccine design and assisted in the development of new vaccine designs. This review summarizes the relevant literature focusing on studies using structural biology to reveal and define HIV-1 Env sites of vulnerability; to improve Env trimers, by creating more stable versions; understanding antibody responses in preclinical vaccination studies at the atomic level; understanding the glycan shield; and to improve "on-target" antibody responses versus "off-target" responses.

Methods: The authors conducted a narrative review of recently published articles that made a major contribution to HIV-1 structural biology and vaccine design efforts between the years 2000 and 2021.

Discussion: The field of structural biology is evolving at an unprecedented pace, where cryo-electron microscopy (cryo-EM) and X-ray crystallography provide complementary information. Resolving protein structures is necessary for defining which Env surfaces are accessible for the immune system and can be targeted by neutralizing antibodies. Recently developed techniques, such as electron microscopy-based polyclonal epitope mapping (EMPEM) are revolutionizing the way we are analysing immune responses and shed light on the immunodominant targets on new vaccine immunogens. Such information accelerates iterative vaccine design; for example, by reducing undesirable off-target responses, while improving immunogens to drive the more desirable on-target responses.

Conclusions: Resolving high-resolution structures of the HIV-1 Env trimer was instrumental in understanding and improving recombinant HIV-1 Env trimers that mimic the structure of viral HIV-1 Env spikes. Newly emerging techniques in structural biology are aiding vaccine design efforts and improving immunogens. The role of structural biology in HIV-1 vaccine design has indeed become very prominent and is unlikely to diminish any time soon.

Keywords: broadly neutralizing antibodies; reverse vaccinology 2.0; structure-based vaccine design

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1 | INTRODUCTION

After more than 30 years of extensive research, an effective human immunodeficiency virus 1 (HIV-1) vaccine is still not available. The major challenge for an HIV-1 vaccine is the enormous viral diversity, and an effective HIV-1 vaccine should induce an immune response that could cope with this diversity [1–4]. The only relevant target for vaccine design efforts is the HIV-1 envelope glycoprotein (Env) trimer, the protein that initiates viral entry and the only target for broadly neutralizing antibodies (bNAbs) [5,6].

Env is a trimeric protein complex composed of three gp120 and three gp41 subunits that are held together by weak non-covalent interactions [7,8]. Env is synthesized as a gp160 polyprotein precursor, which is posttranslationally cleaved in its gp120 and gp41 subunits. During synthesis, the protein is decorated by *N*-linked glycans that comprise approximately 50% of its total mass [9–11]. The *N*-linked glycans play various essential roles in the viral life cycle, for example, in protein folding, infectivity by binding to lectin receptors on immune cells and immune evasion [12–17].

Approximately 20% to 30% of people living with HIV (PLHIV) develop bNAbs albeit usually after several years of infection [18]. In recent years, many bNAbs have been isolated and some have remarkable neutralization breadth [19].

Passive immunization studies in rhesus macaques have shown that bNAbs can protect against simian/human immunodeficiency virus (SHIV) infection [20–22]. The protective efficacy of the VRC01 bNAb was tested in two recent randomized clinical trials [23]. The outcome of the trials was that VRC01 was not able to protect against HIV-1 acquisition overall, but did result in a sieving of VRC01-resistant viruses, suggesting that VRC01 was able to prevent transmission of VRC01-sensitive viruses. The implication is that one bNAb specificity will most likely not be enough to induce broad protection, and an effective HIV-1 vaccine will need to induce multiple neutralizing specificities.

Env protein structures are playing an increasingly important role in vaccine design efforts. Resolving protein structures at the atomic level is necessary to define the surfaces that are accessible for the immune system, to determine sites that are targeted by monoclonal antibodies (MAbs), to design immunogens that mimic the proteins found on viral surfaces and to stabilize the desirable conformations [24]. This approach follows from "reverse vaccinology" in which complete genome sequencing of a pathogen is used to select for surface-expressed proteins to be used in a vaccine [25]. The term "reverse vaccinology" was repurposed in 2002 to describe the utilization of antibodies to select or design antigens with the appropriate binding properties [26]. A next iteration, "reverse vaccinology 2.0" involves the use of antibodies and high-resolution structures for the design of vaccine antigens [27].

While these concepts are widely applied in the HIV-1 vaccine design field, such structure-based vaccine design approaches also accelerated the development of novel vaccines against other pathogens. An example includes respiratory syncytial virus (RSV), for which vaccine development was accelerated when the crystal structure of the pre-fusion F glycoprotein was resolved, using the D25 neutralizing antibody (NAb) specific for the pre-fusion form [28,29]. Similar to HIV-1 Env and other class I viral fusion proteins, the RSV F glycoprotein is very unstable and transitions very easily to its post-fusion conformation. The apex targeting D25 MAb stabilized the F glycoprotein in its pre-fusion conformation, resulting in the high-resolution pre-fusion structure of the protein. This structure allowed for the development of a recombinant pre-fusion-stabilized F glycoprotein [24,28]. As structure-guided design is now an integral part of HIV-1 vaccine development, we review here the role of structural biology in defining the sites of vulnerability of HIV-1 Env, and how new vaccine candidates are designed in an iterative manner by using the structural knowledge of immunogens and the antibodies they induce in (pre)clinical immunization experiments. Our focus is on native-like Env trimers, but we will treat other approaches where relevant as well.

2 | METHODS

We conducted a narrative review of the relevant literature focusing on studies that use structural biology to reveal and define HIV-1 Env sites of vulnerability. This information was used to improve soluble Env trimers by several strategies: (a) by creating more stable versions; (b) to understand antibody responses in preclinical vaccination studies at the atomic level; (c) to understand the glycan shield; and (d) to improve "on-target" antibody responses versus "off-target" responses. Peer-reviewed papers and grey literature that reported on the HIV-1 Env structure, structural improvement of the Env trimer, Env glycosylation and clinical studies were identified between the years 2000 and 19 April 2021. We searched through grey literature and keyword searches in PubMed for the following terms: reverse vaccinology, reverse vaccinology 2.0, structure-based vaccine design, HIV-1 vaccine design, HIV-1 Env structures, HIV-1 Env structure-based vaccine design, HIV-1 broadly neutralizing antibodies, HIV-1 Env glycosylation. We also identified studies in the articles' reference lists.

3 | DISCUSSION

3.1 | Structure-based definition of the Env trimer

"Reverse vaccinology" involves the recombinant generation of the NAb-relevant surface proteins. However, creating stable mimics of the native Env trimers was not achieved until 2013 (Table 1). Since then, much progress has been made in developing faithful mimics of the HIV-1 Env spike. A major initial step involved the design of the BG505 SOSIP.664 prototype native-like Env trimer, based on the clade A transmitter/founder virus BG505 [30]. Several modifications were introduced to stabilize the trimer: a disulphide bond ("SOS") between residues 501 in the gp120 subunit and 605 in the gp41 subunit; an Isoleucine-to-Proline ("IP") substitution at position 559 in the first heptad repeat (HR1) of gp41 that prevents a helix from forming, thereby locking the protein in the pre-fusion state; in improved furin cleavage site to facilitate complete precursor cleavage between gp120 and gp41; and the removal of the membrane-proximal, transmembrane and cytoplasmic tail domains [31]. The BG505 SOSIP.664 trimer closely resembled the native viral Env in both structure and antigenicity [31–35]. It also allowed obtaining the first medium-resolution structures of an Env trimer by both cryo-electron microscopy (cryo-EM) and X-ray crystallography techniques. The structures revealed that the gp120 subunits have a globular conformation, which are assembled into a trimer through key interactions in the V1V2 and V3 domains. The gp41 domain forms a pedestal with the gp120 subunits docked on it, combining into a mushroom-like shape for the functional trimer [36–38]. The high-resolution cryo-EM structure of the JRFL native viral Env also confirmed that the BG505 SOSIP.664 trimer closely mimics viral Env [39] (Table 1). The structure and conformational flexibility of SOSIP trimers were also studied using hydrogen-deuterium exchange (HDX) and double electron-electron resonance (DEER) spectroscopy, revealing that these regions are more conformationally flexible than others [40,41].

3.2 | Structure-based definition of targets of vulnerability on the Env trimer

A plethora of bNAbs have been isolated from PLHIV that provide templates for vaccine design according to the approaches defined by "reverse vaccinology 2.0". While structures of bNAbs in complex with gp120 were solved before and

Table 1. Key developments in structure-guided HIV-1 vaccine designs in the past decade

Year	Development	Reference
2013	First structure of a native-like trimer in complex with PG9	Julien *et al.*, 2013 [44]
2013	Development of the BG505 SOSIP.664 trimer	Sanders *et al.*, 2013 [31]
2013	First cryo-EM structure of a native-like Env trimer	Lyumkis *et al.*, 2013 [36]
2013	First crystal structure of a native-like Env trimer	Julien *et al.*, 2013 [37]
2014	First structure of the complete pre-fusion conformation gp41	Pancera *et al.*, 2014 [38]
2014	First dynamics of SOSIP trimers using hydrogen-deuterium exchange analysis	Guttman *et al.*, 2014 [40]
2016	First cryo-EM structure of a native HIV-1 viral envelope	Lee *et al.*, 2016 [39]
2016	Development of the eOD-GT8 germline-targeting inmmunogen	Jardine *et al.*, 2016 [118]
2017	Development of the germline-targeting BG505 SOSIP GT1 trimer	Medina-Ramirez *et al.*, 2017 [125]
2018	Evaluation of site-specific glycosylation on virion-derived Envs	Struwe *et al.*, 2018; Cao *et al.*, 2018 [95,96]
2018	Analysis of conformational dynamics native-like Env trimers using DEER spectroscopy	Stadtmueller *et al.*, 2018 [41]
2018	First in-human phase I clinical trial started with the eOD-GT8 60mer vaccine candidate	Clinicaltrials.gov [136]
2018	First in-human phase I clinical trial started with a native-like Env trimer	Clinicaltrials.gov [137]
2018	Development of electron microscopy-based polyclonal epitope wrapping (EMPEM)	Bianchi *et al.*, 2018 [112]
2020	First in-human phase I clinical trial started with a germline-targeting native-like Env trimer	C1inicaltrials.gov [124]

generated important structural information on some bNAb epitopes, the availability of the SOSIP trimer allowed many more bNAb epitopes to be determined structurally including epitopes requiring quaternary structures such as those located at the trimer apex and the gp120/gp41 interface [42,43]. While at very low resolution, the first structure to be described was that of the bNAb PG9 in complex with the BG505 SOSIP.664 trimer [44]. The development of the BG505 SOSIP.664 trimer also helped in revealing many sites of vulnerability on Env [45,46]. These bNAbs target eight well-defined clusters comprising of (1) the V1V2 quaternary structure-dependent epitope cluster, involving the N156 and N160 glycans and electropositive residues in the C-strand of the V2 [47]. Inducing neutralizing breadth to this epitope can be achieved by a limited amount of mutations, making this an attractive vaccine target [48,49]. The bNAbs targeting this site possess a very long CDRH3, which is required to navigate through the glycan shield. (2) The N332 centred supersite of vulnerability, which can be subdivided into a V3-glycan epitope cluster and an outer domain (OD) cluster, but both involve the N332 glycan [46]. This site is being explored in immunogen design efforts discussed below. (3) The CD4 binding site (CD4bs) is targeted by the most potent bNAbs discovered and a main feature is that bNAbs targeting this site need to accommodate the N276 glycan [50]. Many bNAbs to this site, those belonging to the VRC01-class, are derived from VH1-2 and have a light chain with a short five amino acid CDRL3. (4) The highly glycosylated "silent" face on Env is a relatively newly discovered epitope that comprises of the glycans N262, N295 and N448. Only a few bNAbs against this site have been isolated, but some of them show remarkable breadth, making the silent face an additional target for vaccine design efforts [51,52]. (5) The gp120-gp41 interface comprises of protein domains and glycans of both the gp120 and gp41 subunits and recognize the pre-fusion closed state of Env [43]. (6) The Fusion peptide epitope cluster comprises of domains in gp120, gp41 and the N-terminal part of the fusion peptide (FP) [43]. This epitope cluster overlaps with the previous one. (7) The gp41-domain is targeted by the 3BC176 and 3BC315 bNAbs and these bNAbs neutralize the virus by increasing the decay of the Env trimer [53]. (8) Finally, the membrane proximal external region (MPER) is a highly conserved, hydrophobic region in gp41, and is targeted by bNAbs that are very broad, but have moderate potency [19]. While the above classification of epitopes is useful, in fact, the Env trimer surface appears to form one contiguous target for bNAbs. When multiple bNAbs are plotted on the trimer surface virtually no uncovered surface remains (Figure 1) [46]. Furthermore, while the extensive glycosylation on Env was long thought to create an immunologically "silent face" and act as a "glycan shield" to protect the underlying Env protein domains against NAbs [6,54], the definition of bNAb epitopes reveals that the glycan shield itself is, paradoxically, a target for bNAbs. As some of these clusters are capable of inducing very potent bNAbs, they are of particular interest for vaccine design efforts, and we further discuss below sites that are being explored as potential vaccine targets.

3.3 | Structure-based immunogen improvement

While class I viral fusion proteins are intrinsically unstable, a number of tricks can be applied to stabilize them in their pre-fusion conformation. We briefly review a few important ones that are now routinely used, but there are other possibilities (Figure 2). For a more extensive review, see Torrents de la

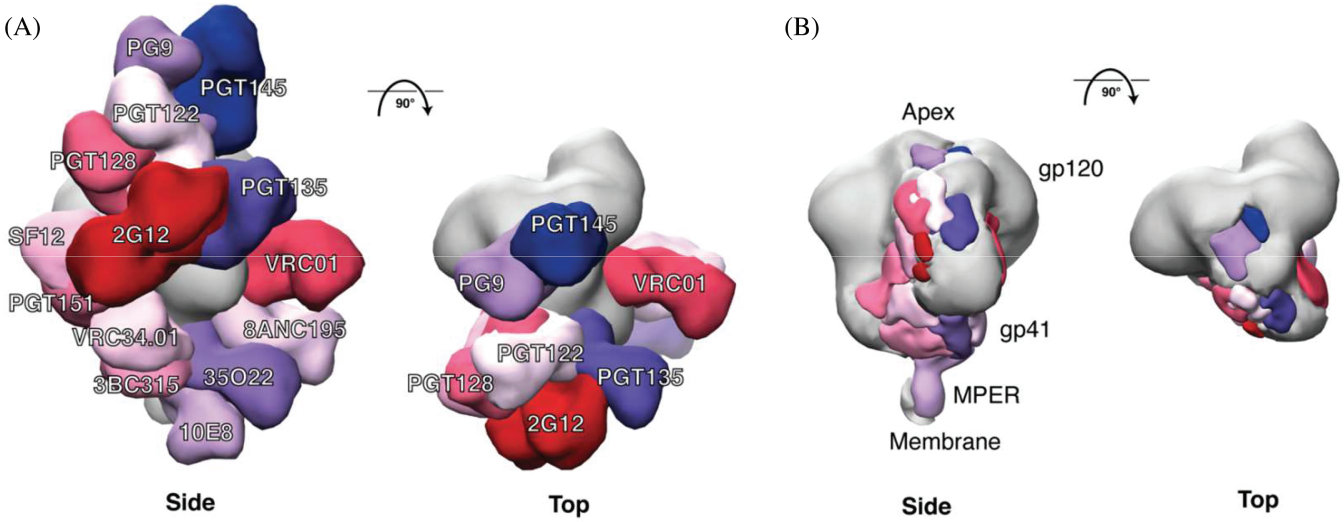


Figure 1. bNAb epitopes mapped onto the three-dimensional structure of the BG505 SOSIP.664 trimer. (a) Side and top views of the bNAbs labelled in different colours that are modelled onto an EM density map of the BG505 SOSIP.664 trimer (coloured in grey). The figure includes bNAbs recognizing eight well-defined sites of vulnerability: PG9 and PGT145 (V2apex), PGT122 and PGT128 (N332-glycan); PGT135 and 2G12 (OD-glycan) both involve the N332 glycan; VRC01 (CD4bs); SF12 (silent face); PGT151, 8ANC195 and 35O22 (gp120-gp41 interface); VRC34.01 (fusion peptide); 3BC315 (gp41); and 10E8 (MPER). Only one copy of each epitope per trimer is shown for clarity. Thus, the model does not indicate the stoichiometry of bNAb binding, only the location of the epitope. (b) Side and top views of the bNAb footprints displayed in (a). This figure is an updated version of fig. 1 from de Taeye *et al.*, 2016 [53] (we thank Gabe Ozorowski for preparing it).

Peña *et al.*, 2018 [55].

3.3.1 | *Trimer stabilization using disulphide bonds*

The initial step of the SOSIP design involved the introduction of a well-placed disulphide bond between gp120 and gp41 described over two decades ago [32]. The structures of the BG505 SOSIP.664 trimer sparked the designs of more stable versions of the Env trimer by adding additional disulphide bonds. Such hyperstabilization of native-like Env trimers can reduce their flexibility and increase their stability, which could be beneficial for promoting interactions of B-cell receptors (BCRs) with bNAb epitopes. Several studies increased the stability of the Env trimer by introducing disulphide bonds [56,57]. The DS-Env trimer, a variant of the BG505 SOSIP.664 trimer, is conformationally fixed by a disulphide bond at positions 201 and 433 that prevents conformational changes associated with CD4 binding, resulting in reduced binding of non-NAbs targeting the V3 and CD4bs [57]. Torrent de la Pena *et al.*, created a hyperstable BG505 SOSIP trimer by introducing several new disulphide bonds, including one between gp120 and gp41 from different protomers covalently linking them, leading to improved stability and antigenicity [58]. A similar approach was used to stabilize the glycoprotein complex (GPC) of Lassa virus (LASV), and involved a disulphide bond to covalently link the GP1 and GP2 subunits [59].

3.3.2 | *Trimer stabilization using prolines*

The second critical component of the original SOSIP design involves a proline substitution in HR1 that prevents conformational changes down the fusion pathway, thereby stabilizing the Env trimers in the pre-fusion state. Proline substitutions at other positions, as well as glycine substitutions, can have similar stabilizing effects [34,60,61]. This method proved to be a universal method for stabilizing class I fusion proteins. Its next use was for the stabilization for RSV F proteins, but applications to LASV GPC [59], Ebola virus GP, and MERS-CoV and SARS-CoV Spike (S) proteins swiftly followed [62–65]. The latter configuration was adopted in SARS-CoV-2 vaccine design, and the Pfizer/BioNTech, Moderna, Curevac, Janssen/J&J, and Novavax COVID-19 vaccines all include the proline stabilization method [66,67].

3.3.3 | *Trimer stabilization using hydrophobic residues*

While the BG505 SOSIP.664 trimer is a good mimic of the native Env spike, it can still fluctuate between a closed and more open state(s). This flexibility can lead to the exposure of the V3 [68–70]. Several strategies to further stabilize the trimer involved further reducing flexibility and silencing of the immunodominant V3. Using structure-guided design, the A316W mutation was introduced to strengthen the hydrophobic interactions at the trimer apex, reducing the propensity of the V3 to "flip out" of its position underneath the V1V2 domain [71]. The introduction of this tryptophan significantly reduced the V3-directed responses in rabbits and mice [71]. By using structure-based design and mammalian display, other hydrophobic residues were introduced between the V3 and V1V2-domains to reduce spontaneous exposure of the V3 by introducing leucine residues at positions 306 and 308 to create hydrophobic interactions with the tryptophan at position 316, by the "so-called" MD39 mutations, in the trimer core

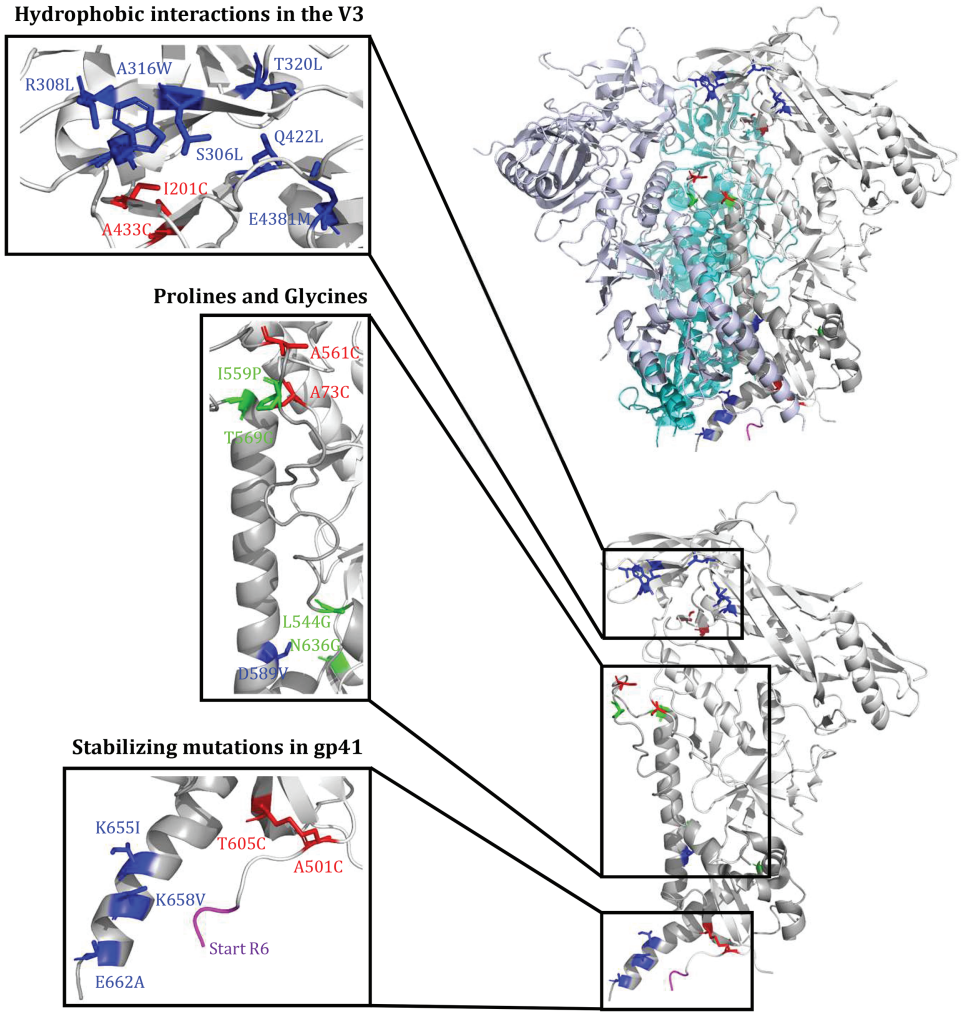


Figure 2. Amino acid substitutions that are routinely used to stabilize soluble native-like trimers. The amino acid substitutions were modelled on the BG505 SOSIP.664 trimer (see text for details). The gp120 subunit is coloured in white and the gp41 subunit in light gray. In gp120: blue - S306L, R308L, A316W, T320L, E381M and Q422L hydrophobic residues; red - I201C-A433C and A73C-A561C disulphide bonds; green - I559P, L544G, T569G and N636G stabilizing mutations; magenta - adjacent residues of the R6 furin cleavage site or flexible linkers. In gp41: red - A501C-T605C disulphide bond; blue - D589V, K655I, K658V and E662A hydrophobic stabilizing mutations. A more extensive list of stabilizing mutations was reviewed previously [55].

(A204I, T320L, E381M, Q422L), or in gp41 (D589V, K655I, K658V, E662A) [72–76].

3.3.4 | *Trimer stabilization by removing the furin cleavage site*

The SOSIP platform includes an improved furin cleavage site to allow for complete precursor cleavage, which requires coexpression of the furin protease. This configuration was also applied successfully to LASV GPC. The BG505 SOSIP structures allowed for the design of single chain (SC) or native flexibly linked trimer (NFL) platforms, in which the furin cleavage site is replaced by a flexible linker allowing for the production of native-like trimers independent of the presence of furin [60,77–79]. These flexible linkers have a specific length to allow for the natural association of the Env subunits, and these platforms often contain some or all of the SOSIP modifications to assist in stabilization. Replacement of the cleavage site in combination with proline stabilization has also aided the development of SARS-CoV-2 vaccines [66].

3.4 | Broadening the panel of native-like trimers

The enormous sequence diversity of HIV-1 is a key challenge in designing an effective vaccine. The vaccine should induce bNAb responses that can cope with this diversity [1–4]. For MAbs to be effective against highly mutable pathogens, they have to undergo a mutation process called somatic hypermutation (SHM), to produce high-affinity MAbs that can tolerate this sequence diversity. Developing immunization strategies that drive this process will be key for an effective HIV-1 vaccine. *In silico* studies revealed that cocktail and sequential immunization regiments could be good strategies to drive the SHM process [80–82]. Therefore, increasing the number of

native-like trimers based on different genotypes would be a good starting point. The high-resolution structures of the BG505 SOSIP.664 trimer helped in the designs of more stable versions of this protein and helped in the identification of residues involved in its inherent stability [31,36–38,58,71,79]. The identification of these residues made it possible to successful apply specific stabilizing mutations to other Env sequences. In recent years, many native-like trimers, based on different platforms, have been developed including ones based on a clade A sequence (92UG037.8), clade B sequences (B41, AMC008, AMC009, AMC011, JRFL), clade C sequences (DU422, ZM197M, CZA97.012, 16055), recombinant B/C sequence (LT5.J4b12C) and consensus sequences [56,57,60,69,71,73–79,83–89]. The generation of native-like trimers from any desired Env sequence was taken a step further by Rutten *et al*., 2018, who devised a universal approach in order to obtain diverse high-quality pre-fusion closed Env trimers [76]. A structure-based screening approach was used to develop a "repair and stabilize" (RnS) method, by removing rare amino acids and optimizing regions critical in the folding process. This approach was applicable to diverse HIV-1 Env trimers in order to improve expression and stabilization [76]. The procedure was improved by the development of a pipeline using an automated process called ADROITrimer. This process combined the DS-SOSIP design with RnS to create a large number of pre-fusion-stabilized Env trimers [90].

3.5 | Structure-based interpretation of the glycan shield

Concurrent with the developments in the Env structural biology field, new mass spectrometry (MS) glycan analysis platforms facilitated the dissection of glycans with unprecedented detail. These techniques, when applied to native-like recombinant trimers, allowed for a detailed characterization of the Env glycan shield, including the composition of each glycan, the level of occupancy at potential *N*-linked glycosylation site (PNGS) and how this affects binding of bNAbs [91].

HIV-1 Env has a particular dense glycan shield and this density imposes steric constrains on early glycan trimming enzymes, leading to the large abundance of underprocessed oligomannose-type glycans [91]. These underprocessed glycans form two specific "mannose patches" on Env: (a) the intrinsic mannose patch (IMP), which is highly conserved among all HIV-1 clades; and (b) the trimer-associated mannose patch (TAMP), which only forms on native-like Env trimers [92]. Glycan density thus influences glycan processing and epitope presentation. This was corroborated when PNGS surrounding the 2G12 epitope were removed, thereby increasing the processing of these glycans, which impacted 2G12 binding. A similar effect was observed for the N160 glycan, which showed increased processing after the removal of the N156 or N197 sites, affecting the binding of apex targeting bNAbs [93]. These examples underpin the importance of understanding the role of glycan networks and glycan composition on conserving bNAb epitopes for HIV-1 Env immunogen design. The glycan density was shown to be important for the induction of neutralizing breadth, as during natural HIV-1 infection viruses that have an intact glycan shield are more prone to induce bNAb responses compared to the ones that have holes in the glycan shield [94]. The BG505 sequence lacks the conserved N241 and N289 PNGS and in the BG505 infant, neutralization breadth was first observed after the introduction of the N241 glycan showing the importance of filling glycan holes [94].

At least equally important from an immunogen design perspective was the quantitative measurement of PGNS occupancy on both viral Envs and recombinant SOSIP trimers, showing that several sites on recombinant trimers are underoccupied [95,96]. Underoccupancy creates artificial glycan holes that are immunogenic, but induce NAb responses that are unable to neutralize the corresponding virus on which the respective sites are removed [97,98]. The importance of artificial glycan holes will be discussed below.

The Env glycans and the glycan shield are conformationally dynamic, much more so than the underlying Env protein domains. A recent study described an approach that combined cryo-EM, computational modelling and MS to visualize the glycan shield structure and dynamics [99]. This approach was able to detect subtle changes in PNGS occupancy, glycan compositions and glycan dynamics that can impact the structure of the glycan shield and epitope accessibility [99]. Further *in silico* studies using molecular dynamics simulations attempted to predict glycan movement for HIV-1 and SARS-CoV-2 [100,101]. A better understanding and visualization of the dynamics of the glycan shield helps to understand how bNAbs cope with the glycan shield and how we need to consider this in vaccine design efforts.

3.6 | Structure-based analysis of Ab responses against native-like trimers

3.6.1 | Mapping Ab responses by traditional serology

The extensive studies on the prototype BG505 SOSIP.664 trimer in rabbit and rhesus macaque immunization studies underlined how the glycan shield - and its holes - shape the NAb response [70]. Serum neutralization experiments with mutant viruses revealed several NAb specificities, but with limited breadth. A major proportion of the autologous Tier-2 NAb responses was directed to the large glycan hole around positions 241 and 289, which is specific to the BG505 strain [70,97]. The serum neutralization experiments also identified neutralizing epitopes residing in the V1 and C3/V5 regions [102–104]. The BG505 SOSIP.664 trimer also induced strong Tier-1 neutralizing responses targeting a linear epitope in the V3 of gp120. The V3 is not exposed on most primary viruses, and these specificities are therefore unable to neutralize such viruses [70]. Finally, the removal of the transmembrane domain and cytoplasmic tail creates a neo-epitope at the trimer base, which is immunodominant [105]. Animal vaccination studies using NFL trimers yielded very similar results as BG505 SOSIP trimers, with strain-specific responses to the N241/N289 glycan hole dominating [106,107].

3.6.2 | Mapping Ab responses by structural analysis of MAbs from immunized animals

Traditional serology studies are sometimes difficult to interpret because the mutations might have allosteric effects.

Furthermore, they do not reveal approach angles, atomic contacts and other structural features. MAbs have been isolated from several animal species immunized with the BG505 trimer [97,102,108–111]. EM analysis of these MAbs in complex with the BG505 trimer revealed that many of the strain-specific NAbs indeed targeted the N241/N289 glycan hole created by the absence of these glycans [97,102]. Other studies identified sites leading to narrow-neutralization breadth, in the C3/V4, C3/V5 and the V1 regions [102,108–111]. While these specificities are unlikely to be amendable to developing neutralizing breadth, occasionally MAbs are found that are. An example is a BG505 trimer-induced MAb that targets the gp120/gp41 interface with significant overlap with the epitope of the VRC34 bNAb [98].

3.6.3 | *Mapping Ab responses by EMPEM*

MAb isolation is a laborious process and it always remains unclear how representative a given MAb is for the overall serological response. In addition, it is difficult to appreciate the totality of the polyclonal response based on a limited set of MAbs. Electron microscopy-based polyclonal epitope mapping (EMPEM) is a recently developed technique that allows for a visual snapshot of the specificities present in serum samples [111,112] (Table 1). The values of the EMPEM technique in delineating serum responses was revealed as it uncovered previously unidentified epitopes (described below), but also confirmed the known N241/N289 glycan hole and base targeting specificities [112]. The newly discovered antibody specificities included ones that target the cleft of the trimer (COT) between two protomers, and one around the N241/N289 glycan hole with no neutralization activity [112]. In macaques, similar responses were observed, but also additional epitopes were identified targeting the fusion peptide, V1/V3 and an epitope in the gp120/gp41 interface [111]. The base specificities are not new, but EMPEM analysis revealed that the base responses were dominating very early after the first prime [112]. The visualization of the different specificities present in one serum is a major asset to the toolkit to analyse antibody serum responses. When performed in sequential samples, it also allows visualizing the evolving antibody specificities over time, in response to different immunizations.

3.7 | Structure-based modification of the glycan shield

3.7.1 | *Filling holes in the glycan shield*

While glycans play important roles in bNAb responses, holes in the glycan shield caused by the absence of PNGS that are present in most primary virus isolates appear to dominate the antibody response after vaccination with Env trimer immunogens. These glycan holes induce NAb responses of narrow breadth [70,97,113]. Filling glycan holes could therefore be a strategy to focus the immune response to more desirable targets. This strategy has been successfully applied to BG505, where the absence of the highly conserved PNGS at positions 241 and 289 in the BG505 sequence creates a large immunodominant hole that induces the majority of the strain-specific NAbs (Figure 3) [70,97]. As the NAb responses to this hole are "dead-end" specificities that cannot be broadened, filling this hole may be a good strategy, and it can easily be achieved by restoring the missing PNGS [102,104,113].

3.7.2 | *Filling artificial holes in the glycan shield*

Glycan holes can also exist by underoccupancy of PNGS, creating artificial glycan holes that can distract the immune system from more desirable targets. Several PNGS are underoccupied on BG505 SOSIP trimers, while they are fully occupied on the parental virus (Figure 3). The fact that holes created by PNGS underoccupancy are immunogenic was revealed by isolation of MAbs from BG505 SOSIP.664 immunized rhesus macaques that target the N611 site. These MAbs can only neutralize when the N611 glycan is removed, indicating that the site is fully occupied on the virus [70,97,98]. High-resolution structures of Env in complex with the N611 targeting non-NAb RM20E1 revealed that the N611 glycan is indeed underoccupied to an extend of approximately 60% [98,114]. A sequon engineering strategy increased the occupancy of several PNGS on the BG505 SOSIP trimer, as well on several other Env isolates, thereby eliminating artificial glycan holes [114]. The strategy exploited observations on model proteins from the mid-1990s that N-X-T sequons have a two to three-fold higher propensity to become occupied compared to N-X-S sequons [115]. The replacement of N-X-S sequons by N-X-T sequons enhanced PNGS occupancy overall, although some site-specific adjustments needed to be made [114]. Increasing PNGS occupancy should reduce off-target responses and steer the immune response to more desirable targets.

3.7.3 | *Covering the trimer base using glycans*

MAb isolation and in particular EMPEM analyses revealed that the trimer base is highly immunodominant after immunization with soluble Env trimers. The truncation of the Env trimer from the membrane, in effect creates a large glycan hole that is highly immunogenic but only induces non-NAbs because it is absent from virus-associated Env (Figure 3). As these responses might also be distracting from more desirable NAb responses, covering this large hole might therefore be necessary. This might not be very straightforward. Kulp *et al.* (2017) addressed this by redesigning a BG505 trimer by swapping the orientation of gp120 and gp41 subunits and placing a linker between the two subunits [73,75]. This linker allowed for the introduction of PNGS to shield the glycan base, resulting in reduced binding of base-targeting non-NAbs [75]. Additional efforts to close this hole on Env-based immunogens are underway and include the presentation of Env on nanoparticles [116,117]. In a similar approach, glycans were used to mask the "backside" of the eOD-GT8 immunogen (see below: CD4 binding site-focused approaches) resulting in the MUT16 immunogen [118,119].

3.7.4 | *Introducing new holes in the glycan shield*

Filling the N241/N289 glycan hole on BG505 SOSIP trimers reduces the responses directed to this specific site. Opening holes elsewhere on the trimer, by knocking-out a PNGS can redirect the immune response to these newly formed holes, providing important proof of concept that this strategy

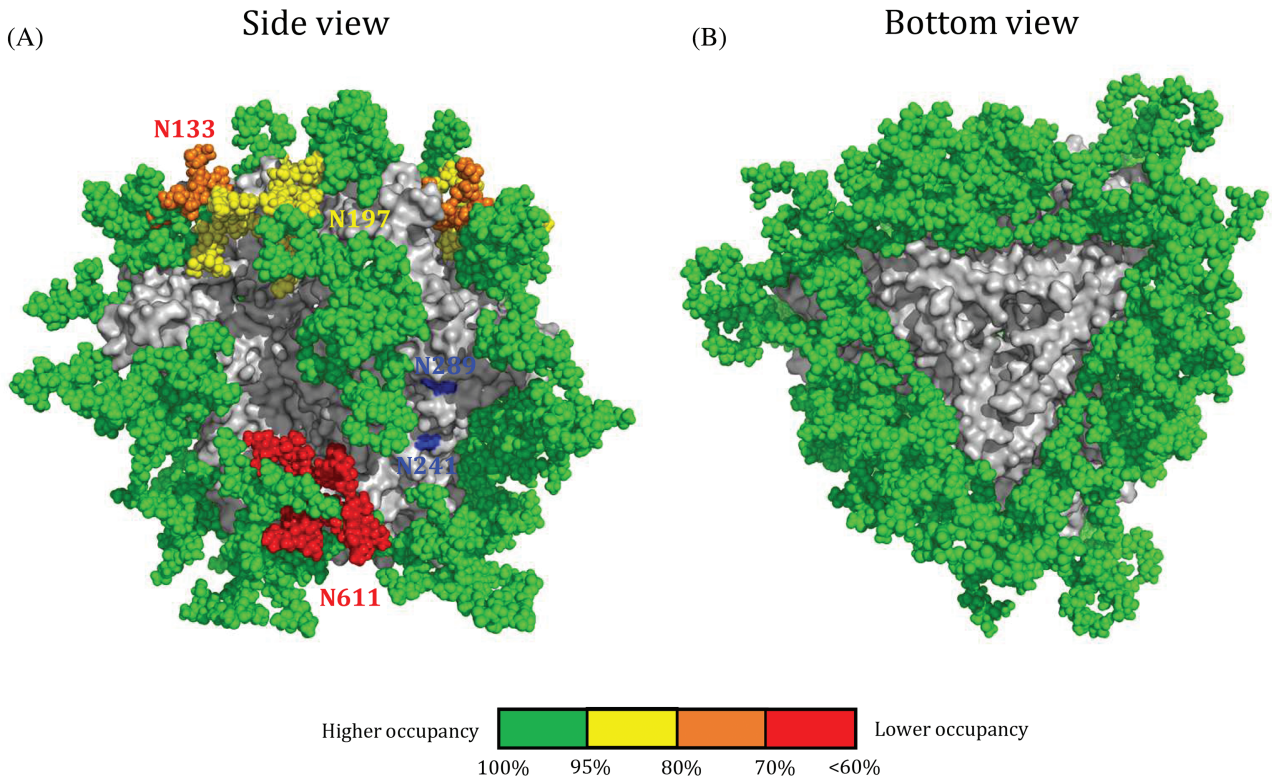


Figure 3. Sources for glycan holes found on stabilized soluble native-like trimers. (a) Side and bottom view of the glycosylated BG505 SOSIP.664 trimer. The glycans were modelled on the crystal structure of BG505 SOSIP.664 (PDB: 5CEZ) using the Glyprot tool from Glycosciences. The missing glycans at positions 241 and 289 on gp120 are indicated in blue. Colour coding reflects the occupancy of each PNGS, similar as described in [114]. Green, full occupancy (>95%); yellow, 80% to 95%; orange, 70% to 80%; red, >60% occupancy. Lower occupancy of a PNGS results in an artificial glycan hole that can be immunogenic. (b) Bottom view of the glycosylated BG505 SOSIP.664 trimer. The removal of the trimer from the membrane creates a large immunodominant hole.

might be valuable [104]. Such strategies are being explored to focus antibody responses to the CD4bs by removing glycans that restrict access to this bNAb epitope cluster [120–122]. In anecdotal cases, this has led to the elicitation of cross-neutralizing antibody responses directed to the CD4bs [120,123,124]. Removing glycans might be particularly relevant for germline-targeting immunogen approaches, that is, approaches based on targeting and activating bNAb precursor B cells with specific genetic signatures [118,119,125]. One component of germline-targeting design often involves enhancing accessibility of the site of interest, while another component involves the structure-based design of modifications to enhance the interaction with desirable naïve B cells.

3.8 | Role of native-like trimer immunogens in driving bNAb responses

In the above sections, we described the development and fine-tuning of native-like trimers, what type of antibody specificities they induce and how they can be further modified. However, they are unlikely to induce bNAb responses by themselves, but they form a platform for further, epitope-specific, immune focusing and/or they provide the final "polishing" steps to finish off a sequential vaccination regimen to mature antibody lineages to become broadly neutralizing (see also Williams *et al.* [126]). We review three prominent epitope-focused approaches that involve native-like trimers at some stage in the regimen. The structures of bNAbs in complex with the HIV-1 Env trimer and the delineation of the sites of vulnerability on Env are the basis of immunogen designs that focuses on specific epitopes. Epitope-focused vaccines can be "germline-agnostic" embracing antibodies derived from different genetic germline signatures, or they can target specific germline classes [43].

3.8.1 | *Fusion peptide-focused vaccine approach*

While it has long been thought that the FP was hidden inside the interior of the pre-fusion Env trimers and was poorly immunogenic, the highly conserved FP became a prominent target following the discovery of the FP targeting bNAbs VRC34 and ACS202 [127–129]. Xu *et al.* investigated the ability of a simple FP peptide to prime FP-directed Ab responses, choosing the eight *N*-terminal residues of the FP as these where shown to be contacted by VRC34 [127,129]. Structure-based design was used to engineer FP-containing immunogens that comprised epitope scaffolds and native-like Env trimers. The FP scaffolds were created by conjugating the eight *N*-terminal residues, as well as the N88 glycan that contributes to the VRC34 and ACS202 epitopes to keyhole

limpet haemocyanin (KLH) as a carrier [129]. An immunization scheme evaluated in nonhuman primates with five FP-KLH primes followed by three trimer boosts, led to neutralizing breadth in a few animals, showing that FP epitope-focused vaccine design merits further study [129].

3.8.2 | *N332 supersite-focused approaches*

The relatively conserved V3-glycan "supersite" is also an interesting target, as very potent bNAbs are induced against this epitope. Furthermore, the majority of PLHIV produce glycan-dependent bNAbs to this epitope [130]. The PGT121-class bNAbs, targeting the N332 supersite in the V3, belong to the most potent and well-characterized bNAbs described today. Steichen *et al*. designed a gl-targeting gp120 molecule that specifically binds PGT121 gl-reverted MAbs, by using mammalian display-directed evolution [73]. These mutations were then transferred to a stabilized BG505 trimer, resulting in a trimer termed $11MUT_B$. Multivalent display of these trimers on liposomes enhanced the activation of B cells that express gl-PGT121 as their BCR, compared to the soluble trimers, and elicited MAbs with neutralization breadth in a KI-mouse model [73,131]. The RC1 trimer, based on $11MUT_B$, lacks the N133 and N137 glycans but also the N156 glycan as it was hypothesized that this would further improve binding of PGT121-class precursors [132]. In a further iteration, termed RC1-4fill, PNGS at positions 230, 241, 289 and 344 were introduced to fill glycan holes (see above) and focus the immune response further to the N332 supersite in the V3. The designs were able to activate and expand B cells in mice, rabbits and macaques that resemble the human V3-glycan "supersite" targeting germline-MAbs [132].

3.8.3 | *CD4 binding site-focused approaches*

The CD4bs is an interesting site for epitope-focused vaccine design because some of the most potent bNAbs target this functionally conserved site. The VRC01-class of CD4bs bNAbs have received much attention in this regard because VRC01-class bNAbs have been identified in multiple PLHIV, and they have distinct genetic signatures that include a VH1-2-derived heavy chain and a light chain with a short five amino acid CDRL3. Designing immunogens that could activate gl-VRC01-class B-cell precursors *in vivo* might be key in developing an effective vaccine that can induce bNAbs targeting the CD4bs. Native-like trimers, such as SOSIP trimers, do not readily induce CD4bs-directed MAbs very easily nor do they interact with gl-VRC01-class MAbs. Reasons are steric constraints because of the location of the CD4bs in the cleft between two protomers, steric hindrance by the N276 glycan and specific atomic contacts with the gl-MAbs [133,134]. The development of the VRC01 lineage was most likely initiated by viruses that lacked the N276 glycan [135]. Several immunogens have been designed to activate precursor VRC01-class B cells. These immunogens include an engineered outer domain (eOD-GT8) and the BG505 SOSIP GT1 trimer [118,125,136]. An updated version of this trimer (BG505 SOSIP GT1.1) entered a phase I clinical trial, as did eOD-GT8 that showed very encouraging results [124,137,138]. In addition to germline targeting approaches for the CD4bs, other germline agnostic approaches involved the deletion of glycans to approach accessibility of the CD4bs [120–122].

4 | CONCLUSIONS

The development of the native-like BG505 SOSIP.664 trimer and its first high-resolution structure fueled the field of Env structure-based vaccine design. The Env trimer structures allowed for the identification of modifications to improve the stability, antigenicity and yield of native-like trimer vaccine candidates, but also allowed for the development of native-like trimers based on different isolates and clades. Native-like SOSIP trimers have now entered phase I clinical trials[124,139–144] (Table 1; Kim *et al*. [145]), and the outcome of these trials will inform whether native-like Env vaccine candidates will induce autologous Tier-2 NAb responses in humans. High-resolution structures of bNAbs and their germline counterparts in complex with native-like HIV-1 Env trimers aided in the identification of their epitopes. New emerging techniques, such as EMPEM, allowing for an increasing understanding of what immunogens induce, will further guide iterative vaccine design efforts with increasing speed. This information will help in reducing undesirable off-target responses and improve driving on-target responses towards the development of bNAbs. Finally, rationally designed nanoparticle platforms, not reviewed here, facilitate the multivalent presentation of Env trimers, thereby increasing B-cell activation and enhancing the magnitude of the antibody response.

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COMPETING INTERESTS

The International AIDS Vaccine Initiative (IAVI) has previously filed a patent relating to the BG505 SOSIP.664 trimer: U.S. provisional application 61/772,739, entitled "HIV-1 Envelope Glycoprotein", with R.W.S. among the co-inventors.

AUTHORS' CONTRIBUTIONS

RD: Writing, original draft preparation and figure preparation. RWS: Writing, review and editing.

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REVIEW

Strategies for induction of HIV-1 envelope-reactive broadly neutralizing antibodies

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Abstract

Introduction: A primary focus of HIV-1 vaccine development is the activation of B cell receptors for naïve or precursor broadly neutralizing antibodies (bnAbs), followed by expansion and maturation of bnAb B cell lineage intermediates leading to highly affinity-matured bnAbs. HIV-1 envelope (Env) encodes epitopes for bnAbs of different specificities. Design of immunogens to induce bnAb precursors of different specificities and mature them into bnAb status is a goal for HIV-1 vaccine development. We review vaccine strategies for bnAb lineages development and highlight the immunological barriers that these strategies must overcome to generate bnAbs.

Methods: We provide perspectives based on published research articles and reviews.

Discussion: The recent Antibody Mediated Protection (AMP) trial that tested the protective efficacy of one HIV-1 Env bnAb specificity demonstrated that relatively high levels of long-lasting serum titers of multiple specificities of bnAbs will be required for protection from HIV-1 transmission. Current vaccine efforts for induction of bnAb lineages are focused on immunogens designed to expand naïve HIV-1 bnAb precursor B cells following the recent success of vaccine-induction of bnAb precursor B cells in macaques and humans. BnAb precursor B cells serve as templates for priming-immunogen design. However, design of boosting immunogens for bnAb maturation requires knowledge of the optimal immunogen design and immunological environment for bnAb B cell lineage affinity maturation. BnAb lineages acquire rare genetic changes as mutations during B cell maturation. Moreover, the immunological environment that supports bnAb development during HIV-1 infection is perturbed with an altered B cell repertoire and dysfunctional immunoregulatory controls, suggesting that in normal settings, bnAb development will be disfavoured. Thus, strategies for vaccine induction of bnAbs must circumvent immunological barriers for bnAb development that normally constrain bnAb B cell affinity maturation.

Conclusions: A fully protective HIV-1 vaccine needs to induce durable high titers of bnAbs that can be generated by a sequential set of Env immunogens for expansion and maturation of bnAb B cell lineages in a permitted immunological environment. Moreover, multiple specificities of bnAbs will be required to be sufficiently broad to prevent the escape of HIV-1 strains during transmission.

Keywords: bnAb precursors; broadly neutralizing antibodies (bnAbs); Env immunogen design; HIV-1 vaccine strategies; HIV-1 vaccines; improbable mutations

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1 | INTRODUCTION

A goal of a highly effective HIV-1 vaccine is to generate broadly neutralizing antibodies (bnAbs) targeting the envelope (Env) surface protein in order to prevent HIV-1 infection — a global health priority. However, bnAbs have features of autoreactive antibodies, thus making them disfavoured for production by host immunity — as discussed in detail in previous reviews [1–3]. Additionally, HIV-1 is a rapidly mutating RNA retrovirus that integrates into the host genome within ~72 h after infection, and once integrated, forms a latent reservoir of infected cells that resists immune system elimination [4–6]. Thus, a successful HIV-1 vaccine must have long-lived, high levels of protective immunity that effects sterilizing immunity and completely prevents infection — a high bar that no other vaccine has had to reach.

Vaccine-induced neutralizing antibodies have been effective against many viruses [7], and serum neutralizing antibodies have been shown to correlate with protection from autologous infection in animal models of HIV-1 infection [8]. BnAbs target susceptible Env sites that are generally conserved across geographically diverse HIV-1 strains and are thus required via vaccination to prevent the acquisition of infection [9,10]. A recent structural survey of HIV-1 Env

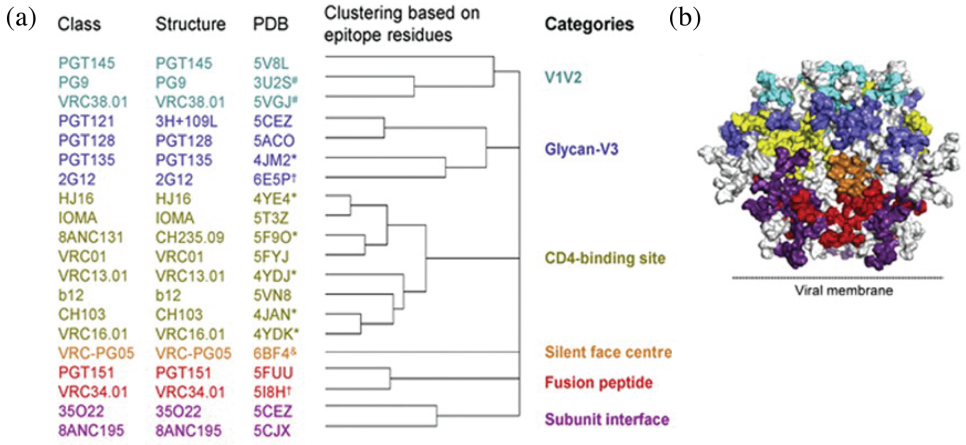


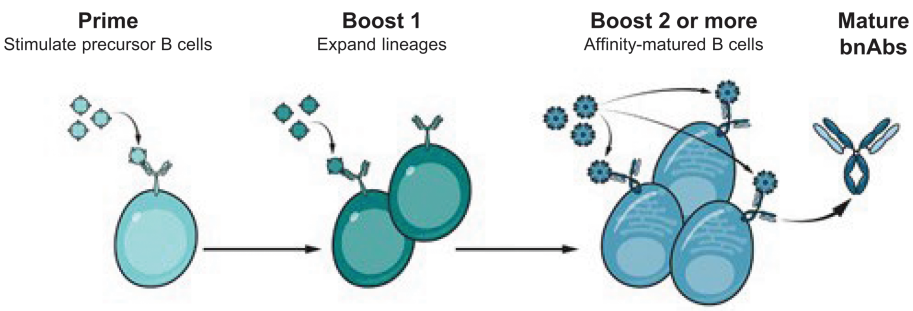
Figure 1. Classification of HIV-1 Env-reactive bnAbs. (A) Twenty classes of bnAbs that recognize the prefusion-closed Env trimer have been segregated into six categories based on their Env residues of interaction. An additional class of bnAbs that target gp41 membrane proximal external region (MPER) (12) is not shown. All HIV-1 Env-antibody complex structures were assigned to classes; leftmost column lists the name of first reported antibody in each class. Each antibody class was categorized based on similarities in B cell ontogeny and mode of recognition. The protein data bank (PDB) IDs shown are for the structures of representative Env-Ab complexes for Abs within each class. The PDBs were chosen based on resolution and degree to which Env in the structure resembled prefusion-closed trimer as described (11). (*) indicates structures determined in deglycosylated gp120-core context; (&) indicates structures determined in partially glycosylated gp120-core context; (#) indicates structures determined with V1/V2 scaffold; (†) indicates a structure with high resolution peptide- or glycan-antibody complex but lower resolution Env trimer-antibody complex structure. (B) Prefusion-closed Env trimer with molecular surface colored by categories defined in (A). This figure was adapted with permission from Chuang et al. (11).

bnAb targets by Chuang and colleagues described six bnAb epitopes on HIV-1 Env gp120 (Figure 1) [11]. In addition, Caillat and colleagues summarized the structure and function of bnAbs targeting the membrane proximal external region (MPER) of Env gp41 near the gp41-viral lipid bilayer interface [12]. Over 50% of the molecular mass of the HIV-1 Env is comprised of glycans that mask neutralizing epitopes [13–15], and therefore, recognition of glycans is key to bnAb development [16,17]. However, HIV-1 Env has evolved to mimic glycosylated host proteins, resulting in minimal recognition of neutralizing epitopes by the immune system [18]. HIV-1 Env also contains many variable loops that can be immunogenic [19], leading to diverting or "off-target" antibodies that may out-compete neutralizing antibodies [20,21]. Thus, HIV-1 Env composition also complicates the efforts for generating bnAbs.

Previous human HIV-1 vaccine efficacy trials that studied first-generation HIV-1 Env vaccines did not generate bnAbs and showed either modest success [22,23] or no protection [24–26]. Recent studies have implied that the frequency of candidate bnAb precursor B cells that possess the immunogenetics, functional and structural properties to mature into bnAb status is limiting [27–29]. Thus, an effective HIV-1 vaccine will need immunogens to engage and mature rare precursor B cell lineages — concepts known as bnAb lineage vaccine design [30] and germline targeting [29,31]. Using information from antibody-virus co-evolution for immunogen design is termed "lineage-based" vaccine design [30]. Keys to this strategy include inference of a bnAb lineage unmutated common ancestor (UCA) B cell receptor (BCR) and design or usage of an optimal Env immunogen capable of expanding this pool of precursor B cells expressing the UCA BCR [30]. Combining structural and immunological information for certain classes of bnAbs, such as the CD4-binding site (bs) antibodies of the VRC01 class, has led to an approach termed "germline targeting" [29,31,32]. Both concepts incorporate the use of immunogens that engage precursor B cells from which bnAbs affinity mature, but germline targeting begins with the estimation that critical bnAb precursor features are sufficiently common within and among different individuals to make them targetable by immunogens [10]. These vaccine concepts are being investigated in human clinical trials led by the HIV Vaccine Trials Network (HVTN).

During bnAb lineage maturation, bnAbs acquire genetic mutations at sites that are rarely targeted for somatic hypermutation, resulting in a low probability of their occurrence in germinal centres (GCs) during affinity maturation — these mutations were described as improbable mutations that impact antibody structure and affinity for antigen to improve antibody function [33,34]. Improbable mutations have been shown to modulate neutralization breadth of the 8ANC131/CH235 class antibody lineages that target the CD4bs [33,35–37], and the DH270 V3 glycan bnAb lineage [34,36]. Additionally, the germline sequences of bnAbs encode paratope structural features that confer recognition of their conserved epitopes on Env. For example, the VH gene-restricted CD4-mimic bnAbs use specific immunogenetics for HCDR2-mediated recognition of the CD4bs: VRC01 class of antibodies use VH1-2*02 paired with light chain genes bearing CDR3s of five amino acids (aa) in length to avoid steric clashes with Env loop D and facilitate maturation to breadth [38,39], whereas the 8ANC131/CH235 class of antibodies use VH1-46 paired with various light chain genes of a range in CDR3 lengths [39]. BnAbs targeting gp41 MPER demonstrated heterogeneity in heavy and light chain gene pairs [12], but a hallmark of MPER bnAbs is a hydrophobic HCDR3 for effective epitope recognition in a two-step process of

(a)



(b)

HIV Env epitope	Antibodies
CD4 binding site	VRC01, ANC131/CH235
V3-glycan	PGT121, DH270, BG18
V1/V2-glycan	PG9, CH01
FDG	DH851, DH1003
Fusion domain	VRC34
MPER	2F5, 10E8, DH511

Figure 2. Strategies for bnAb induction via vaccination. (a) Sequential immunizations with a priming and boosting immunogens will expand B cell lineages from precursor to bnAb status. (b) Examples of antibodies that target different bnAb epitopes on HIV-1 Env. The goal of a vaccine in the Duke CHAVD program is to induce different specificities of bnAb B cell lineages. This image was modified from [10].

interaction [40]. DH270 V3 glycan bnAb used a functional improbable mutation to displace the Env V1 loop and increase access to the bnAb epitope (N332 and GDIR motif) [34,36]. These studies demonstrate that the most potent bnAbs are restricted in their immunogenetics, have improbable point mutations and/or have long (and sometimes hydrophobic) HCDR3s. Thus, successful HIV-1 Env immunogens will need to select bnAb lineage B cells with restricted immunogenetics and improbable functional mutations that facilitate antibody maturation.

Here, we review strategies for vaccine induction of HIV-1 Env bnAbs, which include multiple different approaches by different researchers. These strategies were informed by preclinical studies in mice and rhesus macaques, and some are currently being tested in humans. Moreover, we highlight immunological barriers that an effective HIV-1 Env bnAb-inducing vaccine must overcome.

2 | METHODS

We referenced 131 research articles and reviews that were published between 2001 and 2021 in peer-reviewed journals, and a press-release in 2021. These references were accessed from PubMed between 10 May 2021 and 6 September 2021 when this review was written and revised. We referenced the initial research articles that report seminal discoveries or concepts discussed in this review. For well-established concepts that are commonly discussed in the field of HIV-1 vaccine development and perspectives, we reference review articles.

3 | DISCUSSION

Many HIV-1 sexually transmitted infections are caused by a limited number of transmitted/founder (TF) viruses [41]. Antibody-virus co-evolution studies have demonstrated an "arms race" between the evolving viral quasi-species and host neutralizing antibody responses such that TF viruses induce autologous neutralizing antibodies, but soon escape them, leading to additional neutralizing antibodies. In some individuals, virus Env proteins evolve such that over multiple virus mutation-antibody neutralization cycles, eventually BCRs are selected with broad reactivity [30]. Thus, successful immunization regimens will likely require sequential immunizations to prime and then boost bnAb lineages with the necessary affinity maturation to recognize Env and potently neutralize HIV [10,29–31] (Figure 2). This complexity of immunogen design is unprecedented, and is requiring small iterative phase 1 experimental medicine clinical studies with fewer participants in collaboration with the HVTN to work out feasible and logistically practical immunization regimens that can induce broad and durable serum bnAb activity [10].

3.1 | Requirements for vaccine-induced antibody protection from HIV-1 acquisition

The AMP trials conducted by the HVTN and HIV Prevention Trials Networks (HPTN) studied at-risk cisgender men and transgender persons in the Americas and Europe (HVTN 703/HPTN 081) and at-risk women in sub-Saharan Africa (HVTN 703/HPTN 081). Participants were randomized to receive infusion of the VRC01 bnAb every 8 weeks at doses

of either 10 or 30 mg/kg, or placebo, over 20 months [42]. Overall, the trial did not prevent HIV-1 infection, but it did answer two important questions for the HIV-1 vaccine development effort [43]. First, passive immunization with VRC01 did protect against acquisition of HIV-1, but only against viruses that were highly sensitive to the antibody. The level of neutralizing antibody found to be protective at the time of the HIV-1 transmission was a relatively high serum ID80 titer of ~1:250 [42], thus implying that high levels of protective antibodies will need to be present at the time of transmission for a bnAb-inducing HIV-1 vaccine to be effective. Secondly, the AMP trial showed that serum neutralization titer, as measured in a standardized pseudovirus assay, may predict protection, thereby providing an important analytic tool for future trials [43].

3.2 | Strategies for induction of BNABS

Retrospective studies in people living with HIV-1 who generate bnAbs have identified immunologic perturbations associated with bnAb induction [44–47]. These studies indicated that a permissive environment for bnAb induction is associated with viral antigen persistence, constant immune activation, dysfunction of follicular regulatory CD4 T cells, reduced natural killer cell function and aberrant B cell repertoire that supports the development of autoreactive bnAbs. Thus, for bnAb induction via vaccination, immunogen strategies will need to overcome these immunological roadblocks [3,48].

Animal models are key for testing immunogen strategies to elicit bnAbs. Knock-in (KI) mouse models that express bnAb UCA VH and VL genes produce B cells bearing bnAb UCA BCRs serve as important tools to determine experimentally whether HIV-1 Env priming or boosting immunogens can either expand bnAb precursors or as boosting immunogens select for affinity matured bnAb intermediate or mature antibodies [49–51]. Recently, the development of a simian-human immunodeficiency virus (SHIV)-infected macaque model of bnAb induction provides an important tool for prospective antibody-Env coevolution studies and serves as a molecular guide to inform vaccine induction of bnAbs [52]. Using the SHIV model of bnAb induction in macaques, we can identify Envs as candidate immunogens for bnAb lineage development, and determine the permissible immunological environment for bnAb induction that may be recapitulated via vaccination regimens. In this regard, identifying adjuvants that support distinct immunological environments will be key to the success of an effective HIV-1 vaccine.

BnAb lineage induction by activation and expansion of bnAb precursor B cells, followed by bnAb lineage maturation are key concepts for vaccine induction of bnAbs. We review strategies for bnAb lineage induction and maturation, including vaccine immunogens that are widely being studied for bnAb lineage induction and maturation. We also review vaccine delivery platforms, including more recently attractive platforms for HIV-1 vaccines. For example, lipid nanoparticles are widely employed in vaccine efforts against multiple infectious diseases, including SARS-CoV-2 [53–56], influenza [57], and respiratory syncytial viruses [57–59]. The Moderna [60] and PfizerBioNTech [61] SARS-CoV-2 vaccines used a

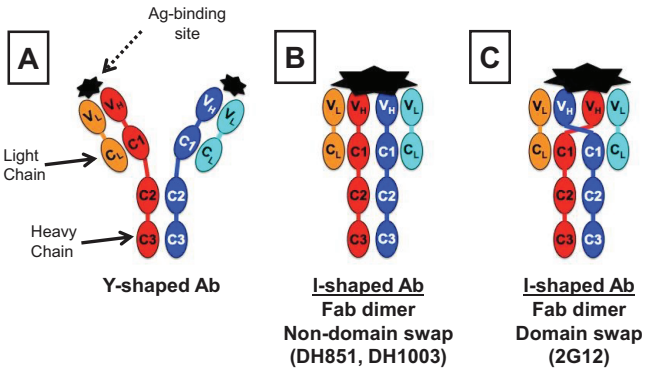


Figure 3. Schematic of antibody (Ab) structures. (a) A canonical Y-shaped Ab has two independent antigen-binding sites. (b) Fab dimerized glycan-reactive (FDG) Ab with VH-VH dimerized I-shaped that has two Fabs acting as a single unit. (c) I-shaped, domain-swapped 2G12 bnAb with an additional binding site formed at the domain-swap interface. Fab-dimerized Abs have a large paratope for Env glycan recognition.

modified messenger ribonucleic acid (mRNA) encoding the SARS-CoV-2 spike protein encapsulated in a lipid nanoparticle as a delivery platform. mRNA-based vaccines have previously shown remarkable success in inducing protective immunity against ZIKA virus (ZIKV) in rhesus macaques [62]. Thus, lipid nanoparticle encapsulating mRNA is an attractive delivery platform for HIV-1 vaccines [63,64].

3.2.1 | *BnAb lineage induction*

To successfully target bnAb precursor B cells, a better understanding of the human naïve B cell repertoire that is capable of Env recognition and maturation into bnAbs is essential [65]. The characteristics of precursor B cells of CD4bs bnAb VRC01, for example, are well-defined, thus facilitating effective repertoire analyses of VRC01 precursors in humans and mice models [27,28,66–68]. For the myriad of bnAb precursors without such well-defined features, more studies are needed to interrogate the immunogenetics, function and structure of these precursor B cells in the naïve B cell repertoire using high affinity antigens designed to bind recombinant bnAb UCA antibodies. Table 1 lists examples of bnAbs with genetic and functional properties that define the selection criteria for candidate precursors of these lineages.

Understanding the population size of precursor B cells will identify attractive targets for vaccination and provides insights into the challenges for inducing bnAb lineages of different specificities. Precursors for bnAbs targeting the Env peptide backbone in conjunction with glycans are rare, approximately 1 in 54 million B cells [29], whereas candidate precursors for bnAbs targeting only Env glycans, termed Fab-dimerized glycan-reactive (FDG) antibodies, are more abundant at 1 in 340,000 B cells [69]. FDG antibodies use a unique VH-VH Fab dimerized conformation to target Env glycans in contrast to the VH domain-swapped conformation used by Env glycan bnAb 2G12 [70] (Figure 3), and we also found FDG B cells with an IgM+IgD+CD27+ marginal zone B cell phenotype [71], suggesting that FDG antibodies originated from the pool

Table 1. Criteria for HIV-1 Env bnAb precursors

BnAb specificity	CD4-binding site	V1V2	Env V3 glycan		MPER
BnAb IDs	CH103, CH235, VRC01	CH01	DH270	2G12, DH851-like	2F5, 4E10, 10E8, DH511
Genetics	[CH103] V_H4-59 + V_L3-1; [CH235] V_H1-46 (W50/ N58/R71); [VRC01] V_H1-2 + 5-aa LCDR3 (V_K1-33, V_K3-20, V_K3-15, V_L2-14)	Long anionic HCDR3; Tyrosine-rich HCDR3; D-D fusion	Long CDRs	Short HDR3; Hydrophobic residues in dimer interface	Hydrophobic HCDR3; Lipid reactivity; polyreactive
Binding	UCA-reactive SOSIP[a,b]; eOD-GT8-reactive[c] CD4bs-KO sensitive	UCA-reactive SOSIP[d] N160-dependent	UCA-reactive SOSIP[e] GDIR-dependent; N332-dependent	Man_9-V3 reactive[f] Glycan-dependent	MPER peptide-reactive[g] D664A/W672A-sensitive
Neutralization	[CH103] CH505.w4.3, CH505TF.gly4; [CH235] CH505 M5_G458Y [VRC01] 426c.TM4/ GnTi-	WITO, Q23, ZM233, T250-4	CH848 10.17 DT; JRFL ΔV1 glycans	Kifunensine-treated HIV-1 isolates	Neutralization of isolates in TZM-bl/FcgR1 assay

BnAb specificities that inform sort strategies for precursor B cell isolation.
[a][CH103] CH505 M11 SOSIPv4.1: WT versus S364K_T455E_G459E (CD4bs KO mutant).
[b][CH235] CH505 M5 SOSIPv4.1_G458Y/GnTi-: WT versus N280D (CD4bs KO mutant) [35,36].
[c][VRC01] eOD-GT8: WT versus N279KD368R (CD4bs KO mutant) [27,38,68].
[d]T250-4 SOSIPv4.1: WT versus N160 (V2 apex KO mutant).
[e]CH848 10.17 DS.SOSIP_N133DN138T: WT versus N332T (V3-glycan KO mutant) [36].
[f]Man_9-V3 glycopeptide: WT versus Aglycone V3 peptide (Env glycan KO mutant) [69].
[g]MPER peptide: WT versus D664AW672A (MPER KO mutant).

of glycan-reactive natural antibodies [72]. FDG precursors were also reactive with yeast glycans, suggesting that high mannose-bearing environmental antigens may prime these B cells, thus increasing their frequencies in the B cell repertoire to respond to glycosylated pathogens. For FDG B cell lineage development, we have identified high mannose-bearing glycopeptide (Man_9-V3) that expanded FDG B cells in macaques and as a bait isolated candidate FDG precursor B cells from HIV-1 naïve individuals. Man_9-V3 Star polymer composed of a 30-mer array of Man_9-V3 glycopeptide [73] is a candidate immunogen for eliciting FDG antibodies in humans; a clinical trial is under development with NIAID Division of AIDS support.

3.2.2 | *BnAb lineage maturation*

GC reactions with persistent, high levels of CD4 follicular-helper T cell (T_{FH}) are required for efficient bnAb B cell affinity maturation [74–78]. In GC reactions, B cells can differentiate into long-lived plasma cells that reside in bone marrow where they secrete and sustain serum antibodies as well as differentiate into memory B cells that disperse and reside both in secondary lymphoid tissues and bone marrow [79,80]. Memory B cells can become reactivated by secondary antigenic encounters leading to sequential rounds of GC maturation [80,81]. Studies to delineate the factors governing B cell fates have provided insights into the role of antigen valencies and affinities for recruiting bnAb precursor B cells into GC reactions [82,83], but careful consideration is needed to choose immunogens with the appropriate structure, valency and affinity for prime and boosting immunogens to select bnAb B cell lineage members via vaccination [84]. A hallmark of bnAb lineage development is the acquisition of functional improbable mutations to be selected in GCs [33–35,85], thus highlighting the need for innovative immunogen design to overcome genetic constraints to bnAb development by selecting functional mutations in bnAb lineages of multiple specificities.

Currently, HIV-1 Env immunogens with varying affinities for bnAb UCAs are being tested in preclinical animal models and human clinical trials for their ability to elicit bnAb B cell lineages via vaccination [28,36,66]. These studies are investigating immunogens capable of priming bnAb precursor B cells and will then evaluate the binding characteristics of these expanded precursors to additional immunogens to identify candidate vaccine boosts. Most bnAb precursors are very rare and a concept is emerging that to expand bnAb precursors, vaccine regimens will need to prime with very high affinity immunogens [28,36,82,83]. However, in studies in mice, it has been suggested that high affinity antigens select for B cells that differentiate into short-lived plasma cells and leave the GC [86]. Thus, for boosting bnAb B cell lineages, tuning the affinity down to a lower level to keep the GC reaction going to favour affinity maturation and selection of BCRs with functional mutations may be required for achieving heterologous neutralization breadth [30,36].

3.2.3 | *Immunization strategies with monomeric Env gp120*

The first antibody response to HIV-1 Env in acute HIV-1 infection is to gp41 that cross-reacts with the gut microbiome

[87–89]. Similarly, immunization with HIV-1 Env in a DNA prime, rAd5 boost regimen in the HVTN 505 trial induced primarily gp41-reactive antibodies that cross-reacted with gut microbiome antigens [90]. The gp41-microbiota cross-reactive antibodies were non-neutralizing and did not mediate Fc-dependent anti-viral functions, thus implicating a role for monomeric Env gp120 immunogens to circumvent a dominant gp41-microbiota cross-reactive B cell responses in vaccination [91]. It has also been hypothesized that immunization of neonates will "imprint" the B cell repertoire such that recognition of otherwise subdominant HIV-1 Env bnAb epitopes will be promoted [92,93]. To address the question whether a gp120 immunogen can prime bnAb precursor B cells in humans, CH505 TF gp120 is being tested in human adults (HVTN115, NCT03220724) and infants (HVTN135, NCT04607408).

We previously isolated a CD4bs antibody of the HCDR3-dominated antibody type [39], referred to as CH103, from an individual chronically living with HIV-1 (CH505) [94]. CH505 TF gp120 induced neutralizing CD4bs antibodies in macaques and clonally expanded CH103 UCA B cells in CH103UCA VH+VL KI mice [95]. Moreover, neonatal macaques and infants are capable of mounting B cell responses against HIV-1 immunogens, including Env gp120 monomers [96,97]. Thus, studying CH505 TF gp120 in human adults (HVTN115, NCT03220724) and infants (HVTN135, NCT04607408) will facilitate evaluation of the B cell repertoires that may support bnAb induction in both human adults and infants. If CH505 TF gp120 elicits bnAb precursor B cells in infants, then these data will provide insights into strategies for bnAb induction in children prior to sexual debut and provide supporting evidence for early life HIV-1 immunization [98].

A modified gp120 protein designed to engage VRC01 germline and intermediate antibodies referred to as 426C core has shown promise in expanding VRC01 precursor B cells in mice when combined with other immunogens [99–101]. Thus, 426C core is another candidate gp120 immunogen that will be tested in human clinical trials for inducing or maturing CD4bs bnAb lineages.

3.2.4 | *Immunization strategies with Env trimers*

A current hypothesis in the HIV-1 vaccine design field is that mimicking the structure of virion-associated Env trimers with recombinant vaccine immunogens will be required for inducing neutralizing antibodies. The rationale behind this hypothesis is that antibodies raised to recognize non-native conformations of Env will lack the ability to bind to fusion-competent native Env on viruses. Recombinant SOSIP trimers were designed as near native-like Env trimers that mainly display bnAb epitopes and have higher affinity for bnAbs than monomeric proteins [102,103]. However, these trimers have a propensity for generating autologous tier 2 neutralizing and gp41 trimer-base binding antibodies in animal models [104–106]. In addition, SOSIP trimer-induced autologous tier 2 neutralizing antibodies can target glycan holes that are vulnerable regions on the trimer that lack potential N-linked glycans [107]. Subsequent reports have shown that glycan hole-targeted antibodies may be difficult to mature to breadth due to strain-specific amino acid differences and the presence of glycans within such epitopes on other HIV-1 isolates [108,109]. Since autologous tier 2 serum neutralizing antibodies elicited by BG505 SOSIP trimer conferred protection to an autologous BG505 SHIV challenge in macaques [8], SOSIP trimers remain under consideration as candidate immunogens in an HIV-1 vaccine, potentially for use as late boosts.

Previously isolated bnAbs have also been used to redesign SOSIP trimers for improved affinities as the next generation of immunogens. For example, BG18 germline and BG18-like precursor antibodies were used to design and select the high affinity Envs N332-GT2 and N332-GT5 [29]. Structural analysis revealed that germline and mature BG18 antibodies demonstrated similar modes of binding to N332-GT2 trimer [29,110]. As an immunogen, N332-GT2 trimer expanded BG18 precursor B cell lineages in BG18 germline-VH KI mice, and as a bait isolated candidate BG18-like human precursor antibodies in humans that shared the same HCDR3 length, D gene, D gene reading frame, D gene position within HCDR3 and JH gene with BG18 [29]. The design of N332-GT2 was based on modifications to BG505 MUT11B SOSIP [111], which was unsuccessful at isolating PGT121-like bnAb precursors [29]. Moreover, modified forms of the BG505 Mut11B SOSIP that were generated by removal of a V1 glycan at position 156 (RC1) in addition to introducing potential N-linked glycan sites at gp120 positions 230, 241, 289 and 344 (RC4-fill) generated serum antibodies and blood-derived B cells with V3 glycan phenotypes, in contrast to BG505 Mut11B trimer [112].

Immunodominant B cell responses elicited by Env oligomers, including SOSIP trimers, are less desirable than bnAbs [113,114], thus understanding how to avoid triggering these cells is important for vaccine induction of bnAb B cells. HIV-1 BG505 SOSIP trimer (HVTN137, NCT04177355) and CH505TF SOSIP trimer (HVTN300, NCT04915768) are being studied in humans; the results of these trials will provide insights into the B cell responses to a near native-like trimer, including antibody responses to bnAb versus non-bnAb epitopes, thereby informing future Env immunogen design.

3.2.5 | *Immunization strategies with multimeric forms of Env oligomers*

Multimeric forms of HIV-1 Env immunogens, including SOSIP trimers, are attractive vaccine candidates for bnAb induction [36,82,83,115]. We and others have shown via antigenic and immunogenicity studies that nanoparticles were desirable for HIV-1 Env bnAb induction [36,57,116–118]. The nanoparticle platform may be more advantageous for uptake by dendritic cells [83,115,119,120], avid BCR engagement and increased antigen presentation that contributes to both enhanced B and T cell responses [64]. Improbable mutations that modulate bnAb lineage development require strong antigenic selection [33–35,85], which may more likely occur with increased antigen presentation conferred by nanoparticle immunogens. Thus, bnAb lineage-inducing HIV-1 vaccine strategies under development by our group use nanoparticles that may be delivered via different platforms.

DH270, a V3 glycan bnAb B cell lineage derived from an individual living with HIV-1 (CH848), provides a blueprint for vaccine induction of a V3 glycan bnAb lineage [34].

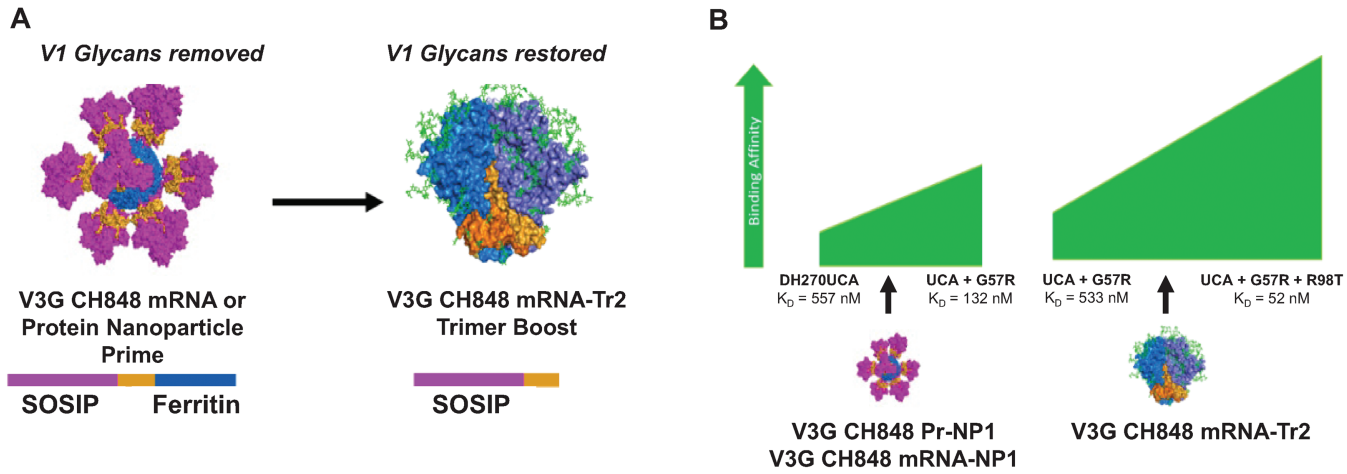


Figure 4. Lineage-based vaccine strategy for inducing V3-glycan bnAbs. (a) Candidate germline targeting priming and boosting immunogens to elicit DH270 V3 glycan bnAb B cell lineages. The priming immunogen will bear Envs with V1 glycans removed, whereas the boosting immunogen will express Envs with the V1 glycans restored. These vaccine immunogens will be formulated as either mRNA in lipid nanoparticles (NP) or as a protein (Pr) followed by a stabilized trimer (Tr) encoded by an mRNA. (b) Priming and boosting immunogens in panel a were selected based on an affinity gradient for recognition of the UCA and mature DH270 bnAb. Immunogen design included modifications of glycans that was informed by structural and functional studies of DH270 UCA and mature bnAb. The following strategy will be studied in humans in collaboration with the HVTN: V3G CH848 Pr-NP1 + mRNA-Tr2.

Co-evolution studies of antibody reactivities and virus sequences from an individual living with HIV-1 (CH848) identified potential Env variants with increasing affinities to the unmutated and affinity-matured DH270 lineage antibodies [34,36]. Importantly, a natural variant of the CH848 TF virus termed CH848 D0949.10.17 was identified that bound to lowly somatically mutated DH270 lineage antibodies. CH848 D0949.10.17 was engineered to remove the N133 and N138 V1 glycans, which enabled nanomolar affinity for DH270 UCA. This immunogen, termed V3G-CH848 Pr-NP1, has been investigated as a candidate immunogen to target the naïve BCRs capable of initiating a DH270-like V3 glycan bnAb B cell lineage [36]. In DH270 UCA KI mice, V3G-CH848 Pr-NP1 formulated as a multimeric nanoparticle protein, indeed, expanded DH270 bnAb precursors and selected for critical antibody heavy and light chain improbable mutations required for heterologous neutralization [11]. The next steps in this mutation-guided, B lineage vaccine design approach are to optimize Env immunogens of appropriate affinities for the bnAb lineage members induced, in order to continue to select for additional functional mutations needed for heterologous virus neutralization breadth — proof of concept for the strategy of targeting naïve V3-glcyan B cells and bnAb lineage-based vaccine design [30] for DH270 V3-glycan bnAb. Similar success in inducing V3-glycan bnAb precursors has been reported by others in V3 glycan bnAb early lineage KI mouse models [29,112,121] and in macaques [112].

Lipid nanoparticle encapsulating mRNA that encode HIV-1 Env has shown promise for eliciting neutralizing antibodies in animal models, some of which were durable responses [102,103] likely due to the intrinsic adjuvant effects provided by the lipid nanoparticles [122]. It remains unknown if the mRNA lipid nanoparticle provides an advantage over conventional adjuvants to generate durable HIV-1 Env antibody responses as observed for 3M-052, a TLR-7/8 agonist [123] or as cationic LNPs for protein immunogens [122] — a focus of future studies. The recent success of the mRNA-based COVID19 vaccines has made this vaccine delivery platform attractive for HIV-1 vaccines [63,124,125]. Moreover, we recently showed that lipid nanoparticle encapsulating mRNA can encode nanoparticles bearing HIV-1 SOSIP trimers that initiate bnAb precursor B cell lineage expansion in bnAb precursor VH + VL KI mice [124]. We have proposed a strategy for DH270-lineage development using either mRNA in lipid nanoparticles (V3G CH848 mRNA-NP1) or protein (V3G CH848 Pr-NP1) as the priming immunogen followed by a stabilized trimer encoded by an mRNA (V3G CH848 mRNA-Tr2) as a boost (Figure 4). The SOSIP-ferritin nanoparticle (V3G CH848-NP) bound the UCA of DH270 V3 glycan bnAb (K_D = 557 nM) and had improved affinity for the DH270 UCA bearing the improbable G57R VH improbable mutation (K_D = 132 nM) [36], suggesting that V3G CH848-NP immunogen may select for improbable mutations in DH270 lineage intermediate bnAb B cells and confer bnAb lineage maturation. Similarly, the SOSIP trimer (V3G CH848-Tr) alone bound DH270 UCA (K_D = 533 nM) and had improved affinities for the DH270 UCA with the G57R VH improbable mutation (K_D = 52 nM). The V3G CH848 priming Env will have V1 glycans deleted and the trimer boost will have the V1 glycans restored, since the DH270 UCA bound with better affinities to V1 glycan-deleted Envs, whereas the DH270 lineage intermediate antibodies and bnAbs learned to accommodate the V1 glycans while accessing the V3 glycan bnAb site using the G57R VH improbable mutation [36]. Both the UCA and mature DH270 had the same angle of approach for epitope recognition [36], thus indicating that structural analysis plays a key role in identifying candidate bnAb precursors. We will use a similar concept to find candidate prime and boost immunogens for bnAb lineages of different specificities. In this regard, we will initially test bnAb UCAs against Envs for mea-

surable affinities, then identify boosting Envs that can bind to the UCA bearing improbable mutations with higher affinity than the UCA alone.

Other candidate multimeric immunogens have also been described for induction of bnAb lineages of different specificities. eOD-GT8 was identified via a structure-based immunogen design strategy as a high affinity germline-targeting antigen for binding VRC01 CD4bs precursor antibodies [66]. Compared to lower affinity versions of eOD antigens, eOD-GT8 was shown to be more effective at isolating VRC01 precursor B cells in HIV-1 naïve humans and has been identified as a priming immunogen for VRC01 precursor B cells in humans [27]. Vaccine regimens for guiding VRC01 precursors into bnAb status must overcome nucleotide insertions and deletions (indels) in the antibody variable regions that occur during the maturation of the natural VRC01 lineage [31,82,99,126]. The fusion peptide domain, a key element in the process of viral entry into host cells, has been recently identified as a vaccine-inducing bnAb target in the Env gp41 subunit [127]. Strikingly, an Env fusion-domain multimer generated via an epitope-focusing immunogen design strategy elicited broadly neutralizing fusion-peptide targeted antibodies that demonstrated 31% breadth in immunized mice [128] and 59% breadth in immunized macaques [129]. Thus, vaccine strategies containing fusion peptide immunogen are of interest for testing in human clinical trials [130]. However, additional boosts to eOD-GT8 and fusion peptide priming immunogens must then be designed to continue to select for affinity-matured antibodies with functional improbable mutations that confer increasing levels of neutralization breadth [29,33].

3.3 | Evaluation of BNAB B cell lineages in HIV-1 vaccine clinical trials

Knowing the optimal fold change increase of vaccine-induced bnAb precursors relative to baseline for subsequent bnAb induction is key to evaluating the success of vaccine strategies being tested in phase I human clinical trials to induce bnAb precursor B cells. Establishing such a criterion for bnAb precursors may be achieved by interrogating the immunogenetics, function and structure of bnAb precursor B cells in blood and other compartments where feasible (Table 1) [27,66]. In addition to immunogenicity, phase I clinical trials evaluate reactogenicity and adverse events in vaccine trial participants to determine the safety of vaccine regimens (see protocols using ClinicalTrials.gov identifier).

HVTN115 (NCT03220724), HVTN300 (NCT04915768) and HVTN133 (NCT03934541) studied bnAb-lineage vaccine design [30] in humans. CH505 TF Env gp120 with a low binding affinity for the CH103 UCA (K_D = 550 nM) is being studied in HVTN115 (NCT03220724) to determine if repetitive boosting with low affinity gp120 in GLA-SE adjuvant can induce CH103 precursor B cells. For comparison, HVTN300 (NCT04915768) will study repetitive boosting of GLA-SE adjuvanted, stabilized CH505 TF SOSIP trimers that have a higher binding affinity to CH103 UCA (K_D = 171 nM) than the CH505 TF gp120. HVTN115 (NCT03220724) is also studying sequential CH505 gp120s to test the hypothesis that CH505 TF gp120 can prime CH103 precursor B cells and sequential immunizations with natural variants of CH505 TF will mature these B cells towards breadth, in a manner that recapitulates viral Env and CH103 antibody co-evolution in an individual living with HIV-1 [94]. For

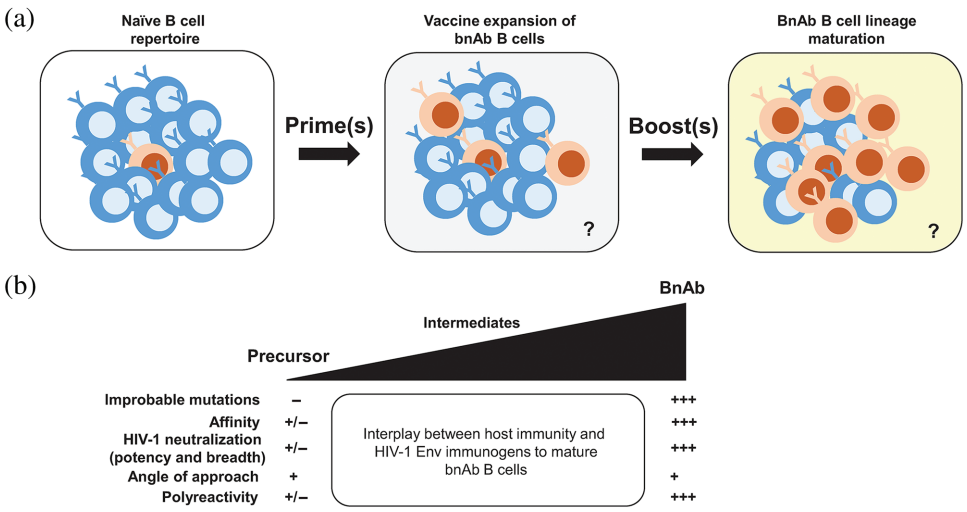


Figure 5. Vaccine engagement and maturation of bnAb precursor B cells in a permissive immunological environment. (a) Expansion of the pool of bnAb precursor B cells in the naïve repertoire following immunizations. In this review, we have discussed various strategies for bnAb lineage expansion that includes priming and boosting immunogens. BnAb lineage expansion in the cartoon is represented by an increase in the frequency of a bnAb B cell lineage (shown in orange) following sequential immunizations (prime and boost). BnAb lineage development will occur in a permissive immunological environment that is not fully established (denoted by question marks). (b) Properties of antibodies during maturation from precursors to intermediate and ultimately bnAb status. Changes in profiles are reflected by +++ symbols over +/– for baseline responses as precursor antibodies.

immune monitoring of HVTN115 (NCT03220724) and HVTN300 (NCT04915768), serum antibodies and the B cell repertoire are being probed for antibodies that bind to CD4bs bnAb epitopes [94,95].

Gp41 MPER liposome was studied in HVTN133 (NCT03934541) as a promising immunogen for eliciting MPER bnAb precursor B cells given that a first-generation gp41 MPER peptide-liposome-containing vaccine regimen expanded 2F5-like B cell precursors and intermediate antibodies in 2F5 bnAb precursor KI mice and rhesus macaques [131]. In HVTN133 (NCT03934541), vaccine trial participants received repetitive doses of gp41 MPER liposome adjuvanted in alum. For immune monitoring of HVTN133 (NCT03934541), serum antibodies and the B cell repertoire are being probed for antibodies that bind to MPER bnAb epitopes [132].

Additionally, eOD-GT8 60mer was tested in healthy volunteers (IAVI G001) as a VRC01 germline-targeting immunogen and expanded B cells with characteristics of VRC01 bnAb precursors (Table 1) in 97% of participants who received the vaccine [133]. This study demonstrated in humans that vaccination with germline-targeting immunogens can achieve success engaging and expanding bnAb precursor B cells of interest in the B cell repertoire. As with all trials that test priming immunogens to elicit bnAb precursor B cells, the next step is to identify the boosting immunogen(s) to mature the B cells to bnAb status.

4 | CONCLUSIONS

A successful bnAb-inducing vaccine strategy will facilitate an interplay between host immunity and HIV-1 Env immunogens to mature bnAb B cells (Figure 5). There are multiple pieces in the puzzle for solving an HIV-1 vaccine to elicit bnAbs: understanding the target (i.e. the naïve B cell repertoire), immunogen design to engage bnAb precursor B cells and select mature B cell lineage members with functional improbable mutations, and vaccine delivery platforms and adjuvants to promote durable antibody responses. Therefore, making an HIV-1 vaccine to induce globally effective, long lasting and safe bnAbs is a daunting task. Global communication and collaboration is essential to complete this difficult task. To accelerate vaccine development, the Collaborative HIV-1 Immunogen Project (CHIP) has been formed, and comprised of the participants of the Duke and Scripps CHAVD, the NIAID Vaccine Research Center, the HVTN, the BMGF, International AIDS Vaccine Initiative and others funded around the world working on HIV-1 vaccines [10]. The goal of CHIP is to promote communication and collaborations such that a successful HIV-1 vaccine can be developed as soon as possible.

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COMPETING INTERESTS

BFH, KW, and KOS have patents submitted on select immunogens and concepts described in this review.

AUTHORS' CONTRIBUTIONS

WBW and BFH drafted the outline and wrote the review. KW and KOS edited the review.

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DATA AVAILABILITY STATEMENT

All the data reference have been published or will be shared in future publications.

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COMMENTARY

Broadly neutralizing monoclonal antibodies for HIV prevention

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Abstract

Introduction: The last 12 years have seen remarkable progress in the isolation and characterization of at least five different epitope classes of HIV-specific broadly neutralizing antibodies (bnAbs). Detailed analyses of these bnAb lineages, maturation pathways and epitopes have created new opportunities for vaccine development. In addition, interest exists in passive administration of monoclonal antibodies as a viable option for HIV prevention.

Discussion: Recently, two antibody-mediated prevention (AMP) trials of a passively administered monoclonal antibody targeting the HIV envelope CD4 binding site, called VRC01, provided proof-of-concept that monoclonal antibody infusion could offer protection against HIV acquisition. While the trials failed to show overall protection against HIV acquisition, sub-analyses revealed that VRC01 infusion provided a 75% prevention efficacy against HIV strains that were susceptible to the antibody. The study also demonstrated that in vitro neutralizing activity, measured by the TZM-bl/pseudovirus assay, was able to predict HIV prevention efficacy in humans. In addition, the AMP trials defined a threshold protective concentration, or neutralization titer, for the VRC01 class of bnAbs, explaining the observed low overall efficacy and serving as a benchmark for the clinical testing of new bnAbs, bnAb cocktails and neutralizing antibody-inducing vaccines. Newer bnAbs that exhibit greater potency and breadth of neutralization in vitro than VRC01 are available for clinical testing. Combinations of best-in-class bnAbs with complementary magnitude, breadth and extent of complete neutralization are predicted to far exceed the prevention efficacy of VRC01. Some engineered bi- and trispecific mAbs exhibit similar desirable neutralizing activity and afford advantages for manufacturing and delivery. Modifications that prolong the serum half-life and improve genital tissue persistence offer additional advantages.

Conclusions: Iterative phase 1 trials are acquiring safety and pharmacokinetic data on dual and triple bnAbs and bi- and trispecific antibodies in preparation for future AMP studies that seek to translate findings from the VRC01 efficacy trials and achieve acceptable levels of overall prevention efficacy.

Keywords: AMP; bnAb; HIV prevention; monoclonal antibody; TZM-bl assay; VRC01

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1 | INTRODUCTION

Despite tremendous effort for the past 30 years to develop an effective HIV vaccine, the field remains challenged. The HIV envelope protein (Env), found on the surface of virions and the major target of neutralizing antibodies, is highly genetically diverse, covered by a glycan shield, and expressed at a relatively low density [1]. Over the last decade, there has been remarkable progress in the isolation and characterization of several different classes of HIV-specific broadly neutralizing antibodies (bnAbs), defined by their ability to neutralize multiple genetically distinct strains. There are essentially five regions of Env that neutralizing antibodies bind: CD4 binding site (CD4bs), variable loop 2 (V2)-apex, V3-glycan, glycoprotein (gp)41/gp120 interface, and membrane proximal external region (MPER) [2–7]. Antibodies targeting all of these sites have been identified, each analyzed for its breadth (number of viral strains it can neutralize) and potency (concentration required for neutralization).

The breadth/potency of these bnAbs fostered the idea of passive administration of monoclonal antibodies (mAb) as an option for HIV prevention, a technique used to prevent respiratory syncytial virus in high-risk infants and, most recently, COVID-19 [8,9]. Advances in next-generation sequencing and B-cell cloning have led to numerous potential bnAbs for efficacy testing. The recently published antibody-mediated prevention (AMP) trials jointly conducted by the HIV Vaccine Trials Network (HVTN) and HIV Prevention Trials Network (HPTN) demonstrated the feasibility of this approach [10]. This commentary focuses on recent results from the first efficacy trials testing bnAbs for HIV prevention and provides a roadmap to move the field forward.

2 | DISCUSSION

2.1 | VRC01 proof-of-concept AMP trials

VRC01, an antibody isolated and characterized from an individual with immunologically controlled HIV, targets the Env CD4bs [11]. VRC01 can neutralize a large percentage of HIV reference strains in vitro using a pseudovirus neutralization assay (see subsequent section) [12,13] and protect against infection in nonhuman primates (NHPs) [14–18]. Several concentrations of VRC01, up to 40 mg/kg, delivered intravenously (IV) were safe and non-immunogenic in HIV-uninfected adults and decreased plasma viral load people living with HIV [19–21]. Recent analyses of participants from a phase 1 trial (who received either 10 or 30 mg/kg VRC01) identified VRC01 in genital tissue, and tissue explants were protected from ex vivo HIV challenge [22]. As such, the proof-of-concept phase 2b AMP studies were designed to test whether VRC01 could prevent HIV infection. AMP participants received either 10 mg/kg VRC01, 30 mg/kg VRC01 or placebo IV every 8 weeks for a total of 10 infusions. These studies were carried out in populations at high risk of acquiring HIV: 2699 men and transgender women who have sex with men in the Americas and Europe (HVTN 704/HPTN 085) and 1924 heterosexual women in sub-Saharan Africa (HVTN 703/HPTN 081) [23,24]. The studies also aimed to establish whether a pseudovirus neutralization assay using TZM-bl cells was a reliable biomarker of HIV prevention and to identify a threshold serum neutralization titer required for protection.

Analyses of the AMP trials indicated that while VRC01 did not provide overall efficacy against HIV acquisition, prespecified analyses indicated a 75% prevention efficacy (PE) against strains that were susceptible to VRC01 [10]. This PE was irrespective of gender, route of transmission, or viral subtype, indicating the innate susceptibility/resistance of the circulating strains to the antibody was the primary determinant of efficacy. While viruses with this level of susceptibility accounted for only 30% of circulating strains at the trial sites [10], the study indicated that bnAbs, when sufficiently potent against infecting viruses, can prevent HIV. As such, subsequent antibodies need to be broader and more potent than VRC01. Another hallmark finding from the AMP trials was that the in vitro TZM-bl neutralization assay was predictive of in vivo HIV sensitivity to antibody. This finding sets the stage for testing new mAbs/mAb cocktails iteratively to down select clinical trial contenders and allow accelerated development of higher potency and more easily manufactured antibodies.

2.2 | Evolution of HIV neutralization assays and reference strains

Neutralization assays with Env-pseudotyped viruses and stable engineered target cells, together with well-characterized reference strains, were transformative and immensely valuable in the discovery and in vitro characterization of bnAbs leading up to the VRC01 AMP trials. A plethora of assays and virus strains used early in the pandemic (1984–2003) produced conflicting results. Attempts to understand these contradictory data pointed to differences in the cells used for virus production and infection target, type of serologic reagent employed (e.g. serum or mAb), and, perhaps most importantly, viral strain used [25,26]. The gold standard assay for many years utilized viruses grown and assayed in peripheral blood mononuclear cells with the assumption that this format best mimicked natural infection; however, these assays were highly variable, low throughput, labour intensive and resisted intensive efforts to standardize. The pseudovirus technology that emerged in 2003 [27,28] afforded superior sensitivity, precision and high throughput capability. Moreover, clonal Envs with distinct sequences enabled detailed functional studies of neutralization epitopes and their escape pathways. A consensus in 2004 emphasized the value of the pseudovirus technology and recommended that well-characterized panels of Env-pseudotyped viruses be developed as standard reference reagents [29]. Subsequently, the Env-pseudotyped virus assay in TZM-bl cells (TZM-bl assay) [30] used in the AMP trials was optimized, qualified, validated [31] and transferred to multiple laboratories around the world [32]. In addition, a formal proficiency testing program was implemented in 2009 to evaluate equivalency of TZM-bl assay performance across multiple laboratories [33]. These efforts improved the accuracy and comparability of results to enable aggregate bnAb datasets to be evaluated with confidence.

Early HIV neutralization studies also suffered from a lack of understanding of the virus. Although a deep appreciation existed for Env sequence diversity, little knowledge was available on the molecular structure and conformational plasticity of native Env trimers. Studies revealed that native-like Env trimers spontaneously transition through open, closed and intermediate conformations, with the open conformation exposing immunodominant epitopes to a greater extent than when the trimer is in an intermediate or closed conformation [34–36]. These conformational states have profound effects on the neutralization phenotype of the virus. An open conformation explains why early studies with T-cell line adapted strains grossly overestimated vaccine-elicited responses [37–39]. Most circulating strains exhibit a more closed trimer conformation that is not susceptible to the bulk of easily induced Env-specific antibodies. To help distinguish these properties, a tiered categorization of HIV-1 isolates was developed based on their neutralization phenotype when assayed with polyclonal HIV-1 sera; from most promiscuously neutralized (open trimer; tier 1; rarely in circulation) to harder-to-neutralize (intermediate/closed trimer; tiers 2 and 3; majority of circulating strains) [35,40].

Hundreds of reference Envs representing all major genetic subtypes and circulating recombinant forms are available and in wide use as pseudotyped viruses for neutralization assays [41–46]. As new bnAbs were discovered and assayed against large panels of reference strains, the magnitude and breadth of bnAb activity was, and continues to be, compared and used to prioritize candidates for clinical development. Testing combinations of bnAbs for possible synergy is also of interest. One large study examined bnAbs targeting four different epitopes (CD4bs, V2-apex, V3-glycan and MPER) in all possible dual, triple and quadruple combinations against a panel of 125 Env-pseudotyped viruses and found the combined effects were mostly additive, being explained by the complementary neutralization profiles of the individual bnAbs [47]. Based on

these additive effects, and using raw data from the study, a Bliss–Hill model was developed that enabled the neutralization magnitude and breadth of various bnAb combinations to be predicted using single bnAb data [48]. Use of the Bliss–Hill model accelerated the identification of optimal bnAb combinations to explore for HIV prevention and treatment without the need for time-consuming and labour-intensive experimental testing of all possible combinations against large panels of viruses [48–50]. Viruses from infected placebo recipients in the AMP trials, representing more contemporary genetic and antigenic diversity, are additional useful reference reagents for single and combination bnAb evaluation.

2.3 | Estimation of protective neutralization titers for future bnAb trials

When the first-in-human VRC01 trials were designed, VRC01 neutralization, measured by the in vitro 50% inhibitory concentration (IC_{50}), surpassed 90% of viral reference strains at an IC_{50} <50 mcg/ml and 72% of strains at an IC_{50} <1 mcg/ml (or 86% at an IC_{80} <50 mcg/ml and 42% <1 mcg/ml) [12,13]. When administered at 10 mg/kg, for example, the in vivo VRC01 trough levels were predicted to be 4 mcg/ml, which corresponds to a neutralization breadth based on IC_{50}/IC_{80} values of 94%/93% against subtype B strains and 80%/73% against subtype C isolates [51]. Results from the AMP trials permitted an estimation of protective serum neutralization titers in vivo (50% and 80% inhibitory dilutions, or ID_{50} and ID_{80}). ID_{50}/ID_{80} titers were calculated by dividing the median mid-infusion VRC01 concentration across the 10 infusions by the IC_{50} or IC_{80} of VRC01 against the participant's acquired virus. Achieving 50%, 75% and 90% PE was predicted to require ID_{50} titers of 1:116, 1:252 and 1:565, respectively, with corresponding ID_{80} titers of 1:32, 1:82 and 1:194 (Gilbert et al. submitted). With this information in hand, it now is possible to design future mAb trials to achieve such neutralizing titers and, thus, clinical efficacy.

2.4 | Comparison of NHP and human data

The rationale for the VRC01 AMP trials was based in part on studies from several laboratories showing that mAbs against multiple Env epitopes could protect against high-dose intrarectal and intravaginal SHIV challenge in NHPs. Early NHP studies showed that neutralizing titers of 50–100 were required for 50% protection [18] and that V2-apex and V3-glycan-specific antibodies protected at remarkably low titers, consistent with their high in vitro neutralizing potencies [52,53]. The AMP study serum neutralization titers now allow validation of NHP challenge models for preclinical predictive efficacy.

To date, there are many similarities between the data derived from NHP high-dose mucosal challenge models and the AMP trials. A meta-analysis of 17 studies involving 274 NHPs and all classes of bnAbs showed that neutralizing activity in serum at the time of challenge was the primary determinant of protection irrespective of challenge virus, route of infection or mAb epitope specificity [54,55]. ID_{50} titers of 1:91, 1:219 and 1:685 were required for 50%, 75% and 95% protection, respectively, which is remarkably similar to titers predicted from the AMP trials mentioned above. These data suggest that neutralization titer in serum has the potential to be a surrogate marker for predicting PE similar to viral load RNA in plasma as a predictor of antiretroviral efficacy. This observation also has important implications for defining the serum titers required for vaccines intended to elicit protective antibodies.

A major priority now is to develop one or more mAb cocktails or multi-specific mAbs that provide high-level (>80%) protection against HIV acquisition in multiple geographic regions and risk groups. This goal is likely achievable, on the basis of the breadth and potency of current mAbs under study and their ability to prevent SHIV infection in NHPs [14,16,18,52,53,56–64]. For example, a recent study showed a mAb cocktail was required for complete protection, as single antibodies failed to protect due to differential resistance profiles of the challenge viruses [62]. These data support the notion that mAb cocktails can enhance PE against diverse HIV strains.

One caveat to using the meta-analysis data is that each NHP experiment used a homogeneous SHIV stock as the challenge virus [54], which is distinct from viral swarms that humans are typically exposed to. In most of these studies, each NHP was administered one bnAb followed by challenge with a single viral strain. Going forward, NHP studies should use strain mixtures to more accurately reflect what occurs during human transmission and provide a better comparative strategy for designing combinations of bnAb cocktails for HIV prevention.

2.5 | bnAbs with greater potency and breadth

As the VRC01 concentration predicted to protect against HIV was insufficient for the majority of circulating strains observed in the AMP trial, future mAbs/mAb cocktails will need wider in vivo coverage and higher potency [63,64]. As with first-generation antiretrovirals, resistance to drug abrogated single drug efficacy and stimulated the use of drug cocktails. The same will most likely be required for mAb prevention strategies.

As the AMP study was ongoing, a series of phase 1 trials were designed and conducted to determine the best antibody or antibody cocktail to move forward clinically. Table 1 lists the current or planned clinical trials for single, double or multiple mAb cocktails, which display a broad range of neutralization potency and breadth [19,65–70]. Great strides have been made in improving upon both breadth and potency [71–73] as well as manufacturability, stability, epitope affinity and serum half-life through antibody screening and targeted engineering [2,74–76]. For example, antibody recycling and turnover is regulated through Fc binding the neonatal Fc receptor, which then limits lysosomal degradation, and modifications to the Fc region can prolong plasma half-life [77]. A leucine and serine double mutation in the Fc region of VRC01, VRC01-LS, increases serum half-life and genital tissue persistence [15,78]. The LS mutation has been subsequently engineered into many anti-HIV bnAbs for clinical trial testing (Table 1).

Table 1. Clinical status of the mAb pipeline for HIV prevention trials

Antibody	Target	Clinical trial completed	Ongoing/in development
VRC01	CD4bs	NCT02568215, NCT02716675, NCT02797171, NCT03831945, NCT02579083, NCT02165267, NCT01993706, NCT01950325, NCT02471326, NCT02664415, NCT02411539, NCT03208231, NCT02463227	NCT02256631, NCT02140255, NCT04860323, NCT04801758, NCT02591420
VRC01-LS	CD4bs	NCT02797171, NCT02599896, NCT02840474	NCT03707977, NCT02256631
VRC07-523-LS	CD4bs	NCT02840474, NCT03205917, NCT03015181, NCT03387150, NCT03565315 (term), NCT03735849,	NCT03928821, NCT04212091, NCT02256631, NCT04357821, NCT04340596, NCT03739996, NCT03721510, NCT03803605
3BNC117 and 3BNC117-LS	CD4bs	NCT02825797, NCT02824536, NCT03571204, NCT02018510, NCT02850016, NCT03254277, NCT02588586, NCT02446847	NCT04811040, NCT04319367, NCT04720742 (susp), NCT03837756, NCT04173819, NCT03588715, NCT03554408, NCT03526848, NCT04250636, NCT03041012, NCT04819347, NCT04560569
CAP256V2LS	V2-apex		NCT04408963
PGDM1400 and PGDM1400-LS	V2-apex	NCT03205917	NCT03928821, NCT03721510
PGT121 and PGT121-LS and PGT121.414.LS	V3-glycan	NCT03205917, NCT02960581	(NCT03928821, NCT03721510, NCT04212091
10-1074 and 10-1074-LS	V3-glycan	NCT02825797, NCT02511990, NCT02824536, NCT03831945, NCT03571204	NCT03928821, NCT03707977, NCT04340596, NCT04811040, NCT04357821, NCT04319367, NCT04720742 (susp), NCT03837756, NCT03619278, NCT04173819, NCT03588715, NCT03554408, NCT03526848, NCT04250636
SAR441236 (Sanofi trispecific)	CD4bs, V2-apex, MPER		NCT03705169)
iMAb/10E8v4	MPER		NCT03875209
10E8-LS	MPER	NCT03565315 (term)	

Note: Trials registered at clinicaltrials.gov. Includes trials in healthy adults, people living with HIV, and HIV-exposed infants.
Studies considered completed based on clinicaltrials.gov.
Abbreviations: Susp, suspended; Term, terminated.

2.6 | Optimizing mAb combinations and delivery

There are several considerations for mAb combination strategies. The potency and breadth of mAb combinations are greater than with single antibodies, double or triple coverage targeting multiple epitopes can impede viral escape, and combinations may reduce levels of incomplete neutralization [47–50]. When tested in vitro against multiclade panels of HIV-1 pseudoviruses, PGT121 and PGDM1400 displayed limited breadth, but also had complementary coverage and outstanding potency, with among the lowest median IC_{50} and IC_{80} titers among all bnAbs identified to date [63].

In addition to using cocktails, the delivery route of antibodies can be altered. IV infusion allows high mAb bioavailability and has no volume limit, but requires clinic visits and is typically expensive. Subcutaneous (SC) injection, on the other hand, could be done virtually anywhere for lower cost, but results in reduced plasma bioavailability compared to IV and is limited by volume (reviewed in [79]). Co-administration with hyaluronidase, however, can increase product uptake with SC administration [79]. Of course, even the most potent bnAbs will not effectively lessen the HIV pandemic unless they are available in areas of most need, typically underserved low- to middle-income countries, with global equitable access [80]. Manufacturing cost, cold-chain requirement, scalability and

portable nature of mAb delivery will need to be optimized for implementation even in low-infrastructure regions (reviewed in [81]).

Bi- and trispecific antibodies have been constructed to broaden neutralization coverage into a single manufacturable antibody. The bispecific antibody 10E8-iMab that targets both Env and host cell CD4 has shown remarkable promise with high potency even at lower concentrations than best-in-class single and combination bnAbs across multiple HIV subtypes [50]. The 10E8 antibody has broad coverage, albeit somewhat lower potency, but when combined with the anti-CD4 mAb ibalizumab, has powerful in vitro activity [82]. A trispecific antibody showing potent activity in NHP [83,84] has also entered clinical trials (Table 1). One of the major unanswered questions of these artificially conceived therapeutics is their uptake in patients and their durability to maintain effectiveness. Monitoring for antidrug antibodies (ADA) will be an important first step in their early evaluation, as they are more "atypical" than human-derived antiviral mAbs, such as VRC01, which has not stimulated ADA in trial participants to date.

3 | CONCLUSIONS

Results from the VRC01 AMP trials and iterative bnAb evaluation over the past decade place us in an opportune time for the future of mAb prevention studies. Our advances in bnAb screening and engineering have drastically increased the potency and breadth of mAbs, identified additional epitopes for neutralization and opened the door for multispecificity and mAb cocktails. Moreover, antibody engineering efforts have generated antibody variants with increased half-lives, potentially enabling administration schedules every 3–6 months and by the SC route, which would be a promising alternative to an antiretroviral-based prophylaxis regimen, especially in regions with low access to IV infusion clinics. In addition, the AMP trials provided critical insights into the serum neutralizing antibody titer required for protection, which will inform the development of next-generation HIV vaccines. It will be of utmost importance for scientists in academia, governmental bodies, commercial partnerships and community stakeholders to collaborate as we build off the first bnAb HIV efficacy trials.

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COMPETING INTERESTS

The authors declare no competing interests.

AUTHORS' CONTRIBUTIONS

LC, DM and MDM developed the outline. MDM wrote the first draft. All authors contributed to writing and editing and approve the final version.

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DATA AVAILABILITY STATEMENT

All data mentioned are provided in this commentary.

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