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# Innovations in HIV-1 Vaccine Design

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## ABSTRACT

**Purpose:** The field of HIV-1 vaccinology has evolved during the last 30 years from the first viral vector HIV gene insert constructs to vaccination regimens using a myriad of strategies. These strategies now include germline-targeting, lineage-based, and structure-guided immunogen design. This narrative review outlines the historical context of HIV vaccinology and subsequently highlights the scientific discoveries during the last 6 years that promise to propel the field forward.

**Methods:** We conducted a search of 2 electronic databases, PubMed and EMBASE, for experimental studies that involved new HIV immunogen designs between 2013 and 2019. During the title and abstract reviews, publications were excluded if they were written in language other than English and/or were a letter to the editor, a commentary, or a conference-only presentation. We then used ClinicalTrials.gov to identify completed and ongoing clinical trials using these strategies.

**Findings:** The HIV vaccinology field has undergone periods of significant growth during the last 3 decades. Findings elucidated in preclinical studies have revealed the importance of the interaction between the cellular and humoral immune system. As a result, several new rationally designed vaccine strategies have been developed and explored in the last 6 years, including native-like envelope trimers, nanoparticle, and mRNA vaccine design strategies among others. Several of these strategies have shown enough promise in animal models to progress toward first-in-human Phase I clinical trials.

**Implications:** Rapid developments in preclinical and early-phase clinical studies suggest that a tolerable and effective HIV vaccine may be on the horizon. (*Clin Ther*. 2020;42:499–514) 

**Key words:** bNAb, HIV vaccine, immune correlates, immunogen.

## INTRODUCTION

Despite decades of research, a tolerable and effective HIV-1 vaccine is still unavailable. The barriers to achieving this goal include overcoming HIV-1 envelope (Env) sequence diversity to rapidly elicit protective broadly neutralizing antibodies (bNAbs) and nonneutralizing humoral and cellular responses.[1–3] In the early years of HIV vaccinology, vaccine development proceeded along traditional pathways that focused on the humoral arm of the immune system[4] as other successful preventive vaccines used antibody binding and function as their correlates of protection.[5] In fact, the classic approach of isolating, inactivating, and injecting whole or partial microorganisms that cause disease had been successfully used since 1796,[6–8] and such approaches were initially attempted for HIV-1. For example, a 1999 mouse study using formaldehyde-inactivated virions was promising,[9] but subsequent analysis found the result to be an artifact.[10] Attenuated HIV-1 was seriously considered as a vaccine candidate until long-term results of naturally occurring attenuated HIV-1 infection, such as the *nef* deleted strain infecting the Sydney Blood Bank Cohort,[11] produced evidence of immunologic damage.[12] A concerted effort for vaccine testing was undertaken by the AIDS Vaccine Evaluation Group and Pediatric AIDS Clinical Trials Group. These consortia, along with several other groups, conducted

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early-phase HIV-1 vaccine studies using recombinant HIV-1 gp160 or gp120 subunit proteins as immunogens. Results of these trials were generally discouraging because they suggested tolerability but not efficacy.[13–15] Regardless, some immunogens proceeded toward efficacy trials as early as 1999.

In the early 2000s, the results from the earliest efficacy trials became available. In particular, the San Francisco–based VaxGen Inc completed 2 Phase IIb/III trials in men who have sex with men and women (VAX004) and intravenous drug users (VAX003). Both studies assessed a protein-based vaccine (AIDSVAX) that contained gp120 proteins from various HIV-1 subtypes.[16,17] The results of VAX004 were again disappointing, with an HIV-1 infection rate of 6.7% in the vaccinated group compared with 7.0% in the placebo group. As in VAX003, there was no demonstrable efficacy, and neither had a significant effect on viral load or $CD4^+$ T-lymphocyte cell counts in those persons who did become infected with HIV-1.[16,17] Despite these discouraging results, there was pressure to advance vaccine science for HIV-1, including the establishment of the Dale and Betty Bumpers Vaccine Research Center (VRC) at the National Institutes of Health in Bethesda, Maryland, and programs to incentivize commercial vaccine development.[18] In this political environment, a decision was made to undertake the RV144 Phase III efficacy trial, which was designed to reassess AIDSVAX in a heterologous prime-boost strategy.[19,20] This trial involved priming the immune system with a canarypox-based vector that contained genetically engineered versions of HIV-1 *env, gag,* and *pol* genes (ALVAC) and boosting with ALVAC and the alum-adjuvanted protein vaccine AIDSVAX. This trial was highly controversial because multiple early-phase clinical trials revealed that the components were poorly immunogenic when given in isolation.[21–25] Proponents argued that the trial provided an opportunity to test the feasibility of the prime-boost design and to test for cellular immune correlates of protection,[22] whereas opponents emphasized the excessive cost of the trial and the high likelihood of failure because of its use of immunogens that had previously induced only modest T-cell and humoral responses with no evidence of broad virus neutralization when administered alone or in combination.[22,23] There was little optimism that this strategy would succeed.

When early vaccine candidates failed to elicit broadly protective antibody responses, the HIV-1 vaccine field shifted its focus to vaccines that would stimulate protective $CD4^+$ and $CD8^+$ T-cell responses. Several animal studies suggested that vaccine strategies that targeted cellular responses might be successful in preventing infection.[26–29] In one such study, simian immunodeficiency virus (SIV)–infected macaques with suppressed SIV replication experienced increased replication when their $CD8^+$ T cells were depleted,[30] suggesting that this subset of T cells was important for controlling viral replication. HIV-infected elite controllers provided further impetus for pursuing this strategy because it was discovered that their impressive viral control was associated with potent and broad cellular responses.[31,32]

This work gave preclinical support for the HIV Vaccine Trials Network (HVTN)–led Phase IIb trials, STEP (HVTN 502) and Phambili (HVTN 503), which were designed to stimulate the cellular response.[33] In both trials, healthy HIV-1–uninfected adults who were considered high risk for HIV-1 acquisition were immunized with a replication-deficient human adenovirus serotype 5 (Ad5) vector with clade B HIV-1 gene inserts (*gag, pol,* and *nef*).[29,34] Interim analysis of the STEP trial revealed higher rates of HIV-1 transmission in the vaccine group compared with the placebo group despite evidence of a robust humoral immune response,[29] leading to early termination of both trials,[34] disappointment in the field,[33] and fear in the volunteer community.[35] Post hoc analysis found that the subgroups of uncircumcised males and persons with preexisting Ad5 neutralizing antibodies had increased HIV-1 infection rates.[36,37]

Two years later, the 2009 report of RV144 trial results restored a sense of optimism because the regimen had a modest vaccine efficacy of 31% at 3.5 years after vaccination, with a vaccine efficacy as high as 61% during the first year.[38,39] However, the findings were controversial because protection occurred despite lack of development of neutralizing antibodies or $CD8^+$ cytotoxic T-cell responses.[38] Nonneutralizing IgG antibodies that targeted the HIV Env variable loops 1 and 2 (V1V2) were identified as correlates of protection, whereas high levels of Env-specific IgA antibodies were associated with a lack of protection.[40] The high cost of these human efficacy

trials combined with the unanticipated results led the field to undertake a more nuanced study of the immunologic response to both vaccination and HIV-1 infection in vaccinees to extract more information from otherwise disappointing studies. These immunologic studies provided clues about which parts of the immune response might be contributing to vaccine-mediated protection. For example, sieve analysis of HIV-1–infected vaccine recipients identified which portions of the immune response appeared to be placing pressure on the virus, and these results have now begun to drive immunogen design.[41–43] Several ongoing efficacy trials are being conducted to better understand the results of the RV144 trial, including HVTN 702 and 705. The HVTN 702 study aims to enroll >5000 participants to assess the tolerability and immune response to ALVAC and bivalent subtype C gp120 adjuvanted with MF59 (ClinicalTrials.gov identifier NCT02968849), and The HVTN 705 study (Imbokodo, Bloemfontein, South Africa) aims to assess a heterologous prime boost regimen using Ad26.Mos4.HIV and Clade C gp140 adjuvanted with aluminum phosphate (ClinicalTrials.gov identifier NCT03060629).

The field also investigated the viral and host factors that contributed to the formation of bNAbs, humoral responses that neutralize multiple HIV-1 clades simultaneously,[44] as well as other mechanisms that might have contributed to protection or altered the course of infection, such as antiviral nonneutralizing antibodies.[45] This work resulted in the isolation of numerous HIV-1 bNAbs from persons living with HIV-1, such as the Vaccine Research Center's VRC01, one of a class of CD4-binding site bNAbs that potently neutralize 91% of known HIV-1 isolates.[46,47] Parallel work on acutely infected persons in which investigators isolated co-evolving bNAbs and HIV-1 isolates resulted in the first generation of germline-targeting vaccine designs[48–50] based on structures of the HIV-1 Env complexed with bNAbs. Potent bNAbs that targeted the membrane-proximal external region of gp41 had even greater neutralization breadth than VRC01,[51–55] and in recent years more potent next-generation bNAbs have produced neutralization breadth as high as 96% of viruses tested.[46,56,57] This understanding has allowed researchers to use the structural conformation of antibodies and their target antigens to guide structure-based immunogen design[58] and use lineage-based vaccine design to target the unmutated common ancestor and intermediate antibodies of bNAb lineages.[59]

The purpose of this narrative review is to describe some of the most innovative HIV-1 vaccine strategies that have emerged during the last 6 years. Given these restrictions, this review is not exhaustive but instead focuses on those immunogens that are currently under investigation in human studies (see Table) or are most likely to advance toward inclusion in Phase I human clinical trials in the near future.

## METHODS

We searched PubMed and EMBASE using the terms *HIV vaccine*, *HIV immunization*, *human vaccine trials*, *SOSIP vaccine, mRNA vaccine, protein vaccine, peptide vaccine, trimer vaccine, AIDS, vaccination, immunization, viral vectors,* and *bNAbs* to capture protein/peptide, mRNA, vector-based HIV vaccine experimental studies and passive immunization strategies. Studies that were published from January 1, 2013, to January 1, 2019, were included in this narrative review. After the initial electronic database search, we identified and removed duplicate publications. The remaining studies then underwent title and abstract review at which time publications were excluded if they were written in a language other than English and/or a letter to the editor, a commentary, or a conference-only presentation. Although quality assessments of the studies were not conducted, all studies included in the full-text review of this study met minimum standards of technical quality and observed protocols mandated by the Animal Welfare Act. We then used ClinicalTrials.gov to identify ongoing clinical trials using these strategies. The complete electronic database search results and citations are listed in the references.

## CURRENT INNOVATIONS

### Native-like Env trimers

The mature, cleaved HIV-1 Env spike is a metastable glycoprotein (gp) heterotrimer of gp120, a surface protein that binds the $CD4^+$ receptor, and gp41, a transmembrane protein involved in fusion of the virion to the host cell.[60] On the basis of the coevolution work that resulted in germline-targeting vaccine designs, the structure of recombinantly

Table. Select clinical trials evaluating HIV-1 vaccine design strategies.

| Clinical trial No. | Immunogen | Estimated end date |
|---|---|---|
| NCT03220724 | CH505TF, week 53, week 78, week 100<br>DNA Mosaic-Tre | 2022 |
| NCT02256631 | VRC01<br>VRC01LS<br>VRC07-523LS | 2021 |
| NCT03699241 | BG505 SOSIP.664 gp140 | 2020 |
| NCT03816137 | ConM SOSIP gp140<br>EDC ConM SOSIP gp140<br>ConS UFO gp140<br>EDC ConS UFO gp140<br>Mosaic SOSIPs gp140 | 2020 |
| NCT02716675 | VRC01 | 2020 |
| NCT02568215 | VRC01 | 2020 |
| NCT03547245 | eOD-GT8 60mer 20 μg + ASO1B/DPBS sucrose | 2020 |
| NCT02824536 | 3BNC117<br>10−1074 | 2018 |
| NCT02362217 | AdCh3NSmut1<br>MVA-NSmut<br>CHAdV63.HIVconsv<br>MVA.HIVconsv | 2017 |
| NCT02366013 | rcAd26.MOS1.HIV-Env | 2016 |
| NCT02315703 | Ad26.Mos.HIV<br>MVA-Mosaic gp140 DP | 2016 |
| NCT02099994 | Ad35-GRIN<br>MVA.HIVconsv pSG2.HIVconsv DNA<br>Electroporated pSG2.HIVconsv | 2015 |

Ad = adenovirus; ConM = consensus sequence of all HIV-1 group M isolates; ConS = consensus sequence; DPBS = Dulbecco phosphate-buffered saline; eOD = engineered outer domain; gp = glycoprotein; MVA = modified vaccinia Ankara; UFO = uncleaved prefusion-optimized; VRC = Dale and Betty Bumpers Vaccine Research Center.

produced Env is thought to be critical to HIV-1 vaccine design because it can display bNAb epitopes in a conformation-dependent manner that may stimulate the bNAb unmutated common ancestors.[45,61] For a vaccine to successfully prevent infection with HIV-1, a virus with high antigenic diversity, it must elicit bNAbs with wide neutralization breadth and/or HIV-1–specific antibodies that mediate antibody-dependent cellular cytotoxicity[62] or other effector functions.[63] Studies have found that HIV-1 virions may be grouped into 4 ranks or tiers based on their pattern of sensitivity to antibody-mediated neutralization, which is associated with the structural configuration of the Env trimer (open, closed, or intermediate).[64] Tier 1A (open) and 3 (closed) viruses are less commonly isolated from infected persons, and these virus isolates have the highest and lowest sensitivity to neutralization, respectively.[64,65] Most HIV-1 strains isolated from acutely infected persons have the tier 2 (closed) phenotype; thus, isolates with that phenotype are the primary targets for immunogen design. The challenge for HIV-1 immunogen design is inducing bNAbs capable of eliciting protection against heterologous

neutralization-resistant (tier 2) viruses or viruses with high resistance profiles.[66]

Various strategies have been used to design stabilized recombinant Env gp140 trimers that mimic the conformation of native Env trimers.[61,67,68] One approach involves stabilizing the gp120-gp41 interactions with an intermolecular disulfide bond (SOS gp140), modified with an isoleucine to proline (I559P) substitution to improve trimerization (SOSIP gp140).[69–71] Researchers designed native-like trimers derived from a subtype A transmitted/founder virus isolated from an HIV-infected infant, BG505.[72–74] Autologous neutralizing antibodies elicited by BG505 SOSIP trimers target epitopes exposed by holes in the glycan shield.[75–77] It is currently unclear whether neutralizing antibodies elicited in this manner may evolve to accommodate glycans in heterologous Envs and develop breadth or if they can be directly elicited by SOSIP immunization. To better elucidate this, researchers are evaluating the immunogenicity of SOSIPs with glycan holes filled.[78] Immunogenicity studies using soluble BG505 SOSIP.664 trimers have found successful bNAb production and neutralization of autologous tier 2 viruses in rabbits,[79] something not previously observed with Env-based immunogens. Currently, a Phase I clinical trial is under way to assess the tolerability and immunogenicity of BG505 SOSIP.664 gp140 in healthy, HIV-1−uninfected participants using a dose escalation strategy. The study is expected to close to accrual in 2020 (ClinicalTrials.gov identifier NCT03699241).

In the past few years, various new trimer designs with enhanced antigenicity and thermostability have been developed.[80–82] One such construct called single-chain gp140 was designed to make the Env cleavage−independent by replacing the cleavage site between gp120 and gp41 with glycine/serine linkers.[83] Another strategy, similar to the single-chained gp140 design, substitutes a flexible glycine/serine linker ($G_4S$) for the cleavage site to yield cleavage-independent Env mimics called native flexibly linked (NFL) trimers.[84,85] The addition of an interpromoter disulfide bond and an L555P substitution in the gp41 heptad repeat region provides improved stability and antigen exposure.[85] Preclinical studies in rabbits, guinea pigs, and nonhuman primates have all found successful autologous tier 2 neutralization responses.[86–88] Manufacturing concerns have previously limited large-scale production of NFL trimers, but a recent study found that these challenges may be overcome with a simplified, large-scale production platform that has successfully produced homogenous BG505 NFL trimers using a Chinese hamster ovary cell line.[89]

The NFL and single-chained gp140 trimer strategies, although promising, have often resulted in fusion intermediate states for uncleaved trimers.[90] To address this and improve stability, researchers replaced the cleavage site with long linkers, resulting in uncleaved prefusion-optimized (UFO) trimers that assume a native-like conformation similar to that of a SOSIP trimer.[90] Furthermore, UFO trimers based on a modified group M consensus sequence called CONSOSL.UFO.750 (stabilized, membrane-bound) and CONSOSL.UFO.644 (soluble) were recently evaluated in preclinical immunogenicity studies. One of the constructs (CONSOSL.UFO.750) had binding to tested bNAbs except for PGT151, whereas the other construct (CONSOSL.UFO.644) induced autologous tier 2 neutralization responses in rabbits after 2 immunizations, although these responses decreased after the third immunization.[91] There are no registered human clinical trials evaluating NFL or UFO trimers at this time, although planning is under way. Although many advances have been made in native-like Env trimer design for vaccine development, there are ongoing hurdles in producing and formulating an ideal construct that is both stable and immunogenic.

## Nanoparticles

In preclinical animal models, nanoparticle HIV-1 immunogens have successfully induced neutralizing antibodies,[88,92,93] activated low-affinity germline precursor B cells,[93–96] and activated follicular T-helper cells.[93,97,98] These next-generation vaccine immunogens are designed to activate and select for rare B-cell precursors with the potential to mature into bNAbs, a strategy called germline targeting.[99] To develop a germline-targeting immunogen prime that binds and activates VRC01-class precursor B cells, Schief et al,[100] at the Scripps Center for HIV/AIDS Vaccine Immunology and Immunogen Discovery, created an engineered outer domain (eOD) of the Env gp120 CD4 binding site. The eOD was simultaneously able to activate select germline-targeting B cells and guide early somatic mutation toward mature bNAb development.[95] The team's

self-assembling nanoparticles displayed 60 copies of the eOD in an effort to improve B-cell activation and lymph node trafficking.[95] The sixth iteration of this germline-targeting construct was successful in activating germline and mature VRC01-class B cells,[95] and a newer variant, germline targeting version 8 (eOD-GT8), had higher affinity for VRC01 class B-cell precursors.[99] In a transgenic mouse model, almost 30% of mice were able to activate VRC01 B-cell precursors and produce a VRC01-class memory response after receiving one immunization using eOD-GT8.[101] Subsequent *in vitro* studies with human samples had the ability of eOD-GT8 to prime the human B-cell repertoire.[102] Because eOD-GT8 has such a high affinity for VRC01-like B cells, it is possible that this immunogen could drive the expulsion of these B cells from germinal centers and force maturation into the short-lived plasma cell compartment. Should this occur, primed B cells would not be able to reenter germinal centers and undergo affinity maturation to develop neutralization breadth. Previous work with eOD-GT2, a lower-affinity version, highlighted potential issues with lower-affinity immunogens because this construct resulted in the development of a substantial endogenous mouse B-cell response that outcompeted the $VRC01^{gHL}$ cells.[103] The eOD-GT8–displaying nanoparticle is currently being evaluated in a Phase I clinical trial sponsored by the International AIDS Vaccine Initiative and is estimated to close to accrual in 2020 (ClinicalTrials.gov identifier NCT03547245).

## B-cell Lineage

There is an increasing consensus that homologous prime-boost immunizations may not be effective in inducing bNAbs against HIV-1[104] because of the protracted evolutionary pathways that bNAbs undergo in HIV-1–infected persons. In addition, some bNAb characteristics suggest that a successful vaccine will need to overcome host immunoregulatory mechanisms that hinder formation of bNAbs, including, but not limited to, down selection of B-cells due to autoreactivity with host antigens.[105] B-cell lineage vaccine strategies re-create the coevolutionary events of the Env and the humoral response by priming the immune system with an early transmitted form of Env and boosting with sequentially evolved Env variants to drive affinity maturation and bNAb development to select for rare lineages.[59] The first of these designs was based on data from an HIV-1–infected individual, CH505, where the co-evolution of the virus and the CH103 CD4-binding site bNAb lineage was observed[106]; this bNAb lineage neutralized 55% of tested HIV-1 isolates and had a lower level of somatic hypermutation compared with other bNAb lineages, making it an attractive vaccine target. The initial vaccine strategy used Env gp120 proteins that reacted most optimally with each step of the CH103 lineage (TF, week 53, week 78, and week 100).[106] This concept is currently being evaluated in HVTN115, a 2-part study designed to assess the tolerability and immunogenicity of the EnvSeq-1 vaccine (CH505TF, week 53, week 78, and week 100) adjuvanted with glucopyranosyl lipid adjuvant formulated in a stable emulsion. In part A, participants were randomly assigned to 1 of 4 groups in a dose escalation test of the initial CH505TF immunogen, whereas in part B, participants are being randomly assigned to additive and sequential Env immunization strategies. This study design complements similar studies previously reported in rabbits[107] and is expected to complete accrual in 2022 (ClinicalTrials.gov identifier NCT03220724).

Further B-cell lineage designs are being pursued based on similar data from other bNAb lineages,[59,108,109] and studies are also evaluating beneficial vaccine outcomes that rely on other antibody functions. For example, the RV144 HIV-1 trial found a reduced risk of infection associated with antibodies that bound Env regions V1V2[40] and neutralizing antibodies to the V2 apex antigenic Env region, meaning that bNAb strategies that target the same region could also have antibody-dependent cellular cytotoxicity or other activities in addition to bNAb activity. Some evidence suggests that this class of bNAbs may be easier to elicit because they are the most prevalent in studies of HIV-1–infected persons, and this class protects macaques challenged with chimeric simian HIV,[110–113] making them attractive vaccine targets.

## RNA-based Vaccines

Nucleic acid–based vaccines, particularly those using mRNA, continue to generate significant excitement as technological advances have recently made this a feasible vaccine strategy.[114] Their benefits include the lack of infectious risk, ability to

modify immunogenicity through different formulations, and ease of manufacturing.[114] Naked mRNA vaccines are not effective because of extracellular RNases that rapidly degrade the genetic material.[114,115] However, chemical and structural modifications to the mRNA and the addition of carrier molecules, including lipid nanoparticles (LNPs), protect the mRNA from degradation and facilitate cellular uptake.[116] This has been used successfully in Zika virus vaccine development,[117,118] and similar mRNA-LNP vaccine designs have elicited potent immune responses to influenza virus in animal and human studies.[119]

One preclinical study found that immunization of humanized mice with low doses of mRNA-LNPs encoding VRC01 bNAbs yielded high levels of protective antibodies against HIV-1 infection.[120] This group later found that a single immunization with a low dose of mRNA-LNP vaccine produced strong $CD4^+$ T cells, in particular T follicular-helper cells, and anti-gp120 IgG responses in mice and rhesus macaques.[121] T follicular-helper cell responses are thought to be critical for eliciting antigen-specific, durable B-cell responses; thus, this vaccine strategy is considered particularly promising.[121] Immunization with self-amplifying mRNA encoding a clade C Env glycoprotein is another mRNA-based vaccine strategy that has produced potent humoral and cellular immune responses in animal models.[122] One research team used a self-amplifying mRNA vector to deliver a mosaic of 6 highly conserved regions of HIV-1 *gag* and *pol* in a mouse model.[123] The results suggested that this method could induce potent and durable $CD4^+$ and $CD8^+$ T-cell responses in those mice who had been primed with the self-amplifying mRNA vector and boosted with a viral vector (modified vaccinia virus Ankara).[123] At this time, Phase I clinical trials are ongoing to assess mRNA vaccines against various infections, including Zika virus and cytomegalovirus, but mRNA-based human vaccine trials have not yet started for HIV-1.

## DNA-based Vaccines

DNA-based vaccines, first developed in the early 1990s, are now experiencing a resurgence because of their excellent immunogenicity in animals and ease of manufacture, scalability, and storage.[124] Early studies revealed that naked DNA plasmid HIV-1 vaccines were poorly immunogenic in humans.[125] However, immunogenicity can now be improved with intradermal delivery using electroporation, a technique that applies transient electrical pulses to cells to increase the permeability of the cell membrane and allow the nucleic acid to enter, or administration in conjunction with molecular adjuvants, such as interleukin 12.[126,127] These adjustments encouraged stimulation of both the cellular and humoral arms of the immune system.[127] HVTN 087 was a Phase I trial designed to assess the effect of increasing doses of plasmid DNA IL-12 adjuvant in conjunction with an HIV-1 multiantigen DNA vaccine delivered via electroporation. The study found that this combination was tolerable and resulted in increased $CD8^+$ T-cell responses but decreased $CD4^+$ responses.[128,129]

## Nonreplicating Viral Vector–based Vaccines

Human Ad5 was used for some early viral vector vaccines, but the cellular and humoral immune responses to vaccine inserts were inhibited by preexisting neutralizing antibodies to the vector.[130] To overcome this challenge, researchers shifted to vectors based on other serotypes with lower seroprevalence, such as Ad26 and Ad35,[131] chimeric forms of adenovirus to prevent immune recognition (Ad5H3) using the antigen capsid-incorporation technique,[132] and the chimpanzee-adenovirus vector to which most humans have not been previously exposed.[133] In the antigen capsid–incorporation strategy, chimeric Ad5H3 was created by adding a polyhistidine sequence ($His_6$) into the hexon3 (H3) capsid protein of Ad5. The resultant chimeric vector was fit and was not neutralized by Ad5 sera, suggesting its ability to overcome the problem of preexisting immunity.[132] The chimpanzee-adenovirus vector strategy has been similarly successful in inducing antigen-specific humoral and cellular immune responses for various pathogens, including Middle East respiratory syndrome coronavirus, Lassa fever, Ebola, and HIV-1.[134–136] There have now been several Phase I clinical trials using an Ad26, Ad35, or chimpanzee-adenovirus vector to deliver HIV-1 vaccines, but none using the chimeric vectors to date.

## Replicating Viral Vector–based Vaccines

Although live attenuated viral vaccines successfully induce high levels of protective antibodies for a number of human infections, including measles and

smallpox,[137] concerns about tolerability have hindered the development of a live attenuated HIV-1 vaccine because they can retain pathogenicity in humans.[138] One attractive alternative that retains the benefits of a replicating viral vector without the risks of HIV-1 infection is recombinant cytomegalovirus (CMV). Unlike other viral vectors, preexisting immunity does not appear to limit the immunogenicity of recombinant CMV vectors.[139] One prototype vaccine using a rhesus CMV vector induced durable SIV-specific effector $CD8^+$ T-cell responses in rhesus macaques and provided aviremic control of infection after mucosal inoculation in 50% of the vaccinees for at least 52 weeks.[140,141] Further analysis revealed that this vector stimulated a unique effector $CD8^+$ T-cell response that recognized diverse epitopes, including those restricted by class II major histocompatibility complex molecules, and had significantly more breadth than that of traditional vaccines.[142] Because human CMV infection of pregnant women can be associated with birth defects, there are concerns about whether this strategy can be made tolerable for wide-scale deployment; thus, this strategy is not yet in human clinical trials.

## Mosaic Vaccines

One hurdle for HIV vaccinology is the design of immunogens that will elicit heterologous neutralization breadth. One approach is a mosaic vaccine in which genetic material from global HIV strains has been computationally analyzed and designed into a polyvalent mosaic immunogen to elicit cellular immune responses against genetically diverse circulating strains of HIV.[143] Mosaic antigens administered by replication-incompetent Ad26 vectors or DNA prime-recombinant vaccinia boost regimens elicit $CD8^+$ T-cell responses and enhance recognition of HIV strain diversity in nonhuman primates.[144] Animals vaccinated with Ad/modified vaccinia Ankara or Ad/Ad vector–based vaccines expressing bivalent HIV-1 mosaic *env*, *gag*, and *pol* had a robust T-cell response, elicited neutralizing and functional nonneutralizing antibodies, and a reduction in acquisition of infection after several mucosal viral challenges.[145,146] Early-phase clinical studies using the mosaic vaccine strategy found strong humoral and cellular HIV-1 immune responses, particularly with mosaic Ad26 as the prime and $Ad26^+$gp140 (high dose) as the boost.[147,148] These studies set the stage for the current efficacy trial, HVTN 706 (MOSAICO), which was designed to assess a regimen of Ad26.Mos4.HIV and adjuvanted Clade C gp140 and Mosaic gp140. Enrollment for this study began in 2019 (ClinicalTrials.gov identifier NCT03964415).

## Passive Immunization

Almost a decade ago, a National Institutes of Health VRC-led research team reported the discovery of 2 broadly neutralizing antibodies, VRC01 and VRC02, which were isolated from a person living with HIV-1.[47] As previously mentioned, this bNAb class neutralized 91% of HIV-1 isolates against which it was tested,[46,47] leading to the launch of a series of Phase I human trials, which found that the infusions were tolerable when received intravenously or subcutaneously every 2–4 weeks.[149] Importantly, VRC01 in participant sera after infusion was active *in vitro* against virus strains.[149] This concept has now transitioned into the antibody mediated prevention (AMP) studies—Phase IIb efficacy trials to assess antibody-mediated prevention, being led jointly by the HVTN and the HIV Prevention Trials Network.

VRC01 was one of the earliest identified and isolated bNAbs with exceptional breadth and potency, but advances in antibody isolation and design are driving new strategies for passive immunization. Mutations have been introduced into VRC01 to alter the amino acid sequence (M428L and N434S) of the constant region of the heavy chain to create VRC01-LS, a form that exhibits a 3-fold longer half-life in serum, longer persistence in mucosal tissue, and 11-fold higher binding affinity to the neonatal Fc receptor without negatively affecting the effector function.[150,151] VRC01-LS was successfully evaluated in a Phase I clinical trial and found to be well tolerated via the subcutaneous and intravenous routes. In addition, because of the extended half-life, VRC01-LS could be administered less frequently than VRC01, making this a more feasible tool for HIV prevention.[152]

Investigators have isolated several additional bNAbs, including, but not limited to, 3BNC117, 10–1074, and 10E8.[153–155] Multiple antibodies that target different epitopes on HIV-1 Env are likely going to be needed for any widely deployable passive immunotherapy program because, much like combination antiretroviral therapy is required to

successfully suppress HIV-1, it has become clear that a single monoclonal antibody will likely not be sufficient to prevent infection.[156] Studies are assessing the safety and pharmacokinetic profiles of infusing combinations of bNAbs for the prevention of HIV-1 infection (ClinicalTrials.gov identifier NCT02824536) and the effects on viral loads in persons living with HIV-1 (ClinicalTrials.gov identifiers NCT02825797 and NCT03526848). However, there are manufacturing and safety challenges that arise when bNAbs are physically combined, so researchers have also explored bispecific and trispecific antibody designs that have the ability to recognize and neutralize several antigenic targets concurrently.[157,158] One group designed 4 bispecific antibodies, of which the combination of VRC07 (which targeted the CD4-binding site) and PG9-16 (which targeted the V1V2 apex) was the most promising, with high potency and a neutralization breadth of 97% of all viruses tested.[157] More recently, a trispecific antibody VRC01/PGDM1400-10E8v4, which targeted the CD4-binding site, membrane-proximal external region, and the V1V2 glycan site, was engineered to explore whether these antibodies could effectively engage multiple effector targets via a 3:1 ratio (trispecific antibody—protein). Trispecific antibodies had higher breadth and potency and neutralized approximately 99% of viruses tested.[159] When evaluated in a rhesus macaque simian HIV challenge model, those animals infused with the trispecific antibodies were 100% protected, in contrast to the 25% protection rate seen with VRC01 alone.[159] In the coming years, we anticipate several additional trials in the antibody-mediated prevention family to test these concepts.

One new strategy is to use a viral vector to transfer bNAb genes directly to the host. Adenoassociated viruses are attractive candidates for this strategy because they are nonpathogenic, integrate into the host genome, and can infect both quiescent and dividing cells.[160,161] Recombinant adenoassociated viruses have already been successfully used to deliver individual bNAbs that protected against repeated viral mucosal challenges in humanized mouse models.[162] Current work is focused on determining the best combination of bNAbs to counter escape mutations and preexisting resistance. As with other infectious diseases, such as diphtheria and hepatitis B where passive immunotherapy and active vaccination were combined to enhance protection, recombinant adenoassociated viruses and other passive therapies combined with active vaccination are likely to become important parts of a successful HIV vaccine strategy.

## CONCLUSIONS

In recent years, our understanding of the host immune response to HIV-1 has become more nuanced and has propelled the field of HIV-1 vaccinology forward. We have learned that manipulating host controls of bNAb induction and displaying antigenic diversity to obtain neutralization breadth are all keys to developing a successful HIV-1 vaccine. One of the most substantial limitations to vaccine development is that we have not yet clearly defined the primary immune correlate(s) of protection against HIV-1 infection, although several potential correlates have been identified, including V1V2-specific IgG antibodies.[40] What we have learned thus far is that the successful vaccine candidate will likely use a heterologous prime-boost strategy that engages both the humoral and cellular immune systems to provide neutralization breadth or include nonneutralization protective immune responses and durability. Even then, such vaccines may be only one piece of a complex puzzle that may involve a combination of active and passive immunization tools.

## DISCLOSURES

The authors have indicated that they have no conflicts of interest regarding the content of this article.

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