New concepts in HIV-1 vaccine development
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With 2 million people newly infected with HIV-1 in 2014, an effective HIV-1 vaccine remains a major public health priority. HIV-1 vaccine efficacy trials in humans, complemented by active and passive immunization studies in non-human primates, have identified several key vaccine-induced immunological responses that may correlate with protection against HIV-1 infection. Potential correlates of protection in these studies include V2-specific, polyfunctional, and broadly neutralizing antibody responses, as well as effector memory T cell responses. Here we review how these correlates of protection are guiding current approaches to HIV-1 vaccine development. These approaches include improvements on the ALVAC-HIV/AIDSVAX B/E vaccine regimen used in the RV144 clinical trial in Thailand, adenovirus serotype 26 vectors with gp140 boosting, intravenous infusions of bNAbs, and replicating viral vectors.

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Introduction
Over three decades after the discovery of human immunodeficiency virus type 1 (HIV-1), a vaccine remains elusive. Variable adherence to combination antiretroviral therapy (cART), limited drug availability, and poor infrastructure are some of the public health roadblocks that show that an HIV-1 vaccine is still needed [1–3]. While cART has resulted in dramatic progress in the treatment of HIV-1 [4], the virus persists in a viral reservoir that is established early in acute infection [5–8], suggesting that there is only a short timeframe within which a vaccine has to be effective. Moreover, enormous HIV-1 diversity makes the design of a successful global vaccine challenging [9–11]. Nevertheless, recent preclinical and clinical vaccine studies have identified several vaccine-induced immune responses that appear to correlate with protection against infection [12*,13–15]. Although these correlates of protection are statistical associations at the present time and may or may not be causal mechanisms of protection [16], they serve as essential guideposts on the pathway of HIV-1 vaccine development and provide scientific hypotheses that the next generation of HIV-1 vaccines will test.

In this review article, we provide an overview of the current HIV-1 vaccine clinical trial landscape, focusing on immune correlates of protection and trials that are underway or in the pipeline. We begin by providing a brief summary of HIV-1 vaccine clinical development from the first efficacy trial, launched in 1998, to the present (Table 1). We then describe several current approaches in HIV-1 vaccine development, each of which is focused on eliciting a different immune response based on immune correlates of protection (Table 2, Figure 1). The first of these approaches is aimed at generating antibody responses specific to the V2 region of the HIV-1 Env protein, with a canarypox viral vector and the Env protein gp120 [17]. The second approach is aimed at generating polyfunctional Env-specific antibody responses, with adenovirus serotype 26 (Ad26) and/or modified vaccinia Ankara (MVA) viral vectors, mosaic HIV-1 sequences, and the Env protein gp140 [18–20]. The third approach is aimed at generating or delivering broadly neutralizing antibodies (bNAbs), exemplified by passive infusion of the CD4-binding site monoclonal antibody (mAb) VRC01 [21,22]. The fourth approach is aimed at generating effector memory T cell responses with the use of live, replicating viral vectors, exemplified by cytomegalovirus vectors.

HIV-1 vaccine clinical development: 1998 – present
To date there have been only 6 HIV-1 vaccine candidates that have reached efficacy trials (Table 1). The early VAX003 and VAX004 trials used the Env subunit protein gp120 to induce immune responses, but the vaccines did not elicit neutralizing antibodies and failed to show efficacy [23–26]. The focus subsequently shifted towards vaccine strategies aimed at inducing cellular immune responses, owing to the prominent role of CD8+ T cell responses in control of HIV-1 replication [27,28]. The Step study (HVTN 502) was the first efficacy trial that focused on cell-mediated immunity using an adenovirus serotype 5 (Ad5) viral vector to express HIV-1 Gag, Pol,
and Nef internal proteins. The Step trial did not confer protection against infection or induce virologic control, and increased rates of infection were observed in a subset of vaccinees [29,30]. The Phambili trial (HVTN 503) tested the same vaccine used in the Step trial and also did not reduce infection rates [31]. The only signal of efficacy of an HIV-1 vaccine candidate was shown in the RV144 trial in Thailand, which showed 31% efficacy with a prime-boost regimen of a canarypox viral vector (ALVAC-HIV) and the Env subunit protein gp120 (AIDSVAX B/E) [17]. The HVTN 505 trial tested a multi-clade DNA prime with a recombinant Ad5 boost designed to generate both humoral and cellular responses, and this vaccine also did not show efficacy [32–34]. The lessons from these trials suggest that the global diversity of HIV-1 strains and the challenge of inducing functional immune responses are major obstacles to a successful HIV-1 vaccine.

Current landscape of HIV-1 vaccine clinical research

Vaccines that elicit V2-specific antibody responses

RV144 used an ALVAC-HIV/AIDSVAX B/E regimen based on clade B and CRF01_AE sequences that matched the prevailing HIV-1 strains in Thailand [17,35]. Subsequent analyses showed that non-neutralizing antibodies to variable loops 1 and 2 (V1V2) regions of HIV-1 Env were associated with a reduced risk of HIV-1 acquisition [12**,15], and a genetic sieve analysis demonstrated that the ALVAC-HIV/AIDSVAX B/E regimen had improved efficacy against viruses that matched the immunogen sequence in the V2 location [15]. Moreover, high levels of antibody-dependent cellular cytotoxicity (ADCC) were also shown to correlate with a reduced risk of HIV-1 acquisition in RV144 [36] and V2-specific monoclonal antibodies isolated from RV144 vaccines were shown to mediate ADCC against HIV-1-infected CD4+ T cells [37]. HIV-1 specific IgG3 responses were also noted to correlate with protection in RV144 and distinguish this trial from the VAX003 trial [13].

These data suggested a path forward for HIV-1 vaccine development by raising the hypothesis that non-neutralizing V2-specific antibodies, particularly if well-matched to local circulating strains, might have the potential to block HIV-1 acquisition (Table 2, Figure 1). As a result, multiple strategies are being pursued to build on the RV144 findings, including:

- Changing the RV144 vaccine schedule to improve the magnitude and durability of V2-specific antibody responses. For example, the RV305 trial is studying extended boosting of the ALVAC-HIV/AIDSVAX B/E regimen in Thailand (NCT01435135).
- Transferring the RV144 regimen to a new region to test whether the V2-specific responses generated in Thailand might also provide protection in a different region. The HVTN 097 trial is testing this strategy by studying the ALVAC-HIV/AIDSVAX B/E regimen in South Africa (NCT02109354); early data indicates similar cellular responses as RV144 [38].
- Adapting the RV144 regimen to a different region to test whether changing the vaccine sequences to match
local prevailing strains will generate regional V2-specific responses. The HVTN 100 trial is pursuing this strategy with testing of an ALVAC-HIV (vCP2438)/bivalent subtype C gp120/MF59 regimen in South Africa (NCT02404311), with plans to move to efficacy testing in the HVTN 702 trial [39].

- **Using different adjuvants to the RV144 regimen** to enhance immune responses [40-42]. For example, HVTN 107 is a planned phase 1/2a study in Zimbabwe comparing ALVAC-HIV (vCP2438)/bivalent subtype C gp120 with either MF59 or aluminum hydroxide [43].

**Vaccines that elicit polyfunctional antibody responses**
The modest and transient vaccine efficacy observed in the RV144 trial has also inspired efforts to push forward with additional vaccine concepts. Polyfunctional Env-specific antibody responses were associated with a significantly reduced risk of simian-human immunodeficiency virus (SHIV) and simian immunodeficiency virus (SIV) acquisition following repetitive rectal challenges in vaccinated nonhuman primates [44**]. Specifically, we showed that rhesus monkeys primed with Ad26 vectors expressing SIVsmE543 Env, Gal, and Pol and boosted with adjuvanted SIVmac32H Env gp140 demonstrated complete protection in 50% of vaccinated animals against a series of repeated, heterologous, intrarectal SIVmac251 challenges that infected all controls. Analysis of antibody Fc functions, including ADCC, antibody-dependent cellular phagocytosis (ADCP), antibody-dependent natural killer (NK) cell activities (ADNKA), antibody-dependent complement deposition (ADCD), and glycosylation profiles, revealed that the protein boost resulted in a more polyfunctional antibody response that also correlated with protective efficacy. These data supported the results of earlier vaccine studies in non-human primates that also demonstrated that non-neutralizing antibody effector functions correlated with protective efficacy [45,46].

**Novel Adenovirus serotype 26 vectors** are thus being evaluated in clinical studies in combination with the Env trimeric protein gp140. In initial studies, an Ad26 vector expressing a clade A HIV-1 Env immunogen (Ad26.Env.A.01) was shown to be safe, well-tolerated, and immunogenic in three completed clinical trials (NCT00618605, NCT01103687, and NCT01215149). Ad26 vectors have been shown in multiple models to be biologically distinct from Ad5 [47], the vector used in the Step study. Ad26 has also been shown to elicit polyfunctional antibody responses in humans using systems serology [48*]. This platform characterized Fc-effector functions, ADCC, ADCP, NK cell IFN-γ secretion, NK MIP-1β secretion, and NK CD107a activation as well as multiple other biophysical measurements [48*]. Using this platform, Chung et al. demonstrated that Ad26.Env.A.01 elicited a network of

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**Table 2**

Ongoing HIV-1 vaccine clinical trials based on known immune correlates of protection

| Vaccines that elicit V2-specific antibody responses | ALVAC-HIV/AIDSVAX B/E in Thailand | NCT01435135 | U.S. Army |
| Testing of RV144 regimen in new region | ALVAC-HIV, AIDSVAX B/E in South Africa | NCT02109354 | HVTN |
| Adapting RV144 regimen to new region | ALVAC-HIV (vCP2438), bivalent Subtype | NCT02404311 | NIAID |
| Changing RV144 adjuvants | ALVAC/gp120 with Alum vs. MF59 | Planned | HVTN |
| None | ALVAC/gp120 | Planned | HVTN |

| Vaccines that elicit polyfunctional antibody responses |
| Novel Vaccine regimens based on Ad26 vector, mosaic immunogens, and gp140 protein | VRC01 | NCT02256631 | NIAID |
| VRC01 | NCT02568215 | NIAID |
| VRC01LS | NCT02598986 | NIAID |
| VRC01 efficacy | Planned |
| None yet | NCT02511990 | Rockefeller Unv. |
| bNAb epitope scaffolds | None yet |
| B cell lineage design | None yet |
| Gen delivery | rAAV1-PG09DP | NCT01937455 | IAVI |

| Efforts that elicit (or deliver) broadly neutralizing antibodies |
| VSV-Indiana HIV Gag | None |
| VSV HIV Gag | None |
| Ad4-EnvC150 | None |
| rvAd26.MOS1.HIV-Env | None |
| HCMV/HIV | None |

HVTN, HIV Vaccine Trials Network; NIAID, National Institute of Allergy and Infectious Diseases; IAVI, International AIDS Vaccine Institute.
Potential correlates of protection for ongoing HIV vaccine studies. (1) V1V2-specific and/or polyfunctional non-neutralizing antibodies (NnAb, left panel). The cartoon represents the mechanism by which non-neutralizing antibodies may bind to infected CD4+ T cells, triggering complement activation, antibody-dependent cellular phagocytosis (ADCP), and/or antibody-dependent cellular cytotoxicity (ADCC). (2) Broadly neutralizing antibodies (nAb, middle panel). The cartoon represents neutralization of the infecting virus via binding to the Envelope glycoprotein. (c) Effector memory T-cells at mucosal sites (right panel). The cartoon depicts continuous antigen stimulation via an effector memory T-cell pool.

Antibody functions and Fc-receptor binding activity that was clearly distinguished from responses elicited by the RV144, VAX003/004, and Step regimens.

Multivalent Ad26 vectors have recently been developed that express mosaic immunogens (Ad26.Mos.HIV) with the goal of generating polyfunctional antibodies and diverse cellular immune responses that recognize HIV-1 epitopes from global viruses. Mosaic HIV-1 Env, Gag, and Pol sequences are bioinformatically designed to optimize coverage of global HIV-1 diversity [49–52], and have been shown to expand cellular and humoral immune responses in non-human primates when expressed by adenoviral vectors [44**,**53–55]. The clinical trial HIV-V-A004/IPCAVD009 (NCT02315703) launched in 2015, is evaluating Ad26.Mos.HIV. MVA vectors expressing the same mosaic antigens (MVA-mosaic) and a clade C gp140 boost. This study is sponsored by Crucell Holland B.V. (Janssen Pharmaceuticals), and has completed enrollment of 400 subjects in Rwanda, South Africa, Thailand, Uganda, and the USA. The MVA-mosaic vaccine was also evaluated in a phase 1 clinical trial, HIV-V-A002/IPCAVD006, that launched in 2014; this study is examining two injections of MVA-mosaic in unvaccinated healthy individuals and individuals who previously received the Ad26.EnvA.01 vaccine (NCT02218125) [18,19]. A third vaccine trial of mosaic immunogens is launching in 2016 and is testing Ad26.Mos.HIV and clade C gp140 using shorter vaccine schedules (HPX1002/IPCAVD010; NCT02685020).

Finally, Env gp140 protein subunit immunogens are also undergoing clinical testing as part of multi-component vaccine regimens to elicit polyfunctional antibodies. For example, the South African AIDS Vaccine Initiative (SAAVI) has begun testing of a Novartis subtype C gp140 boost in the context of a previous DNA/MVA/truncated gp160 vaccine (HVTN 073, NCT01423825 and HVTN 073E, NCT01423825). As mentioned above, a clade C gp140 trimeric protein is also being tested in the HIV-V-A003/IPCAVD008 (NCT02304185), HIV-V-A004/IPCAVD009, and HPX1002/IPCAVD010 studies.

**Efforts to elicit (or deliver) broadly neutralizing antibodies**

One of the unachieved goals in HIV-1 vaccine development is the generation of immunogens that can elicit broadly neutralizing antibodies (bNAbs) [56–58]. Such bNAbs are found in about 10–30% of infected people and develop several years after infection [59]. The sole target of these antibodies is the Env glycoprotein, a highly diverse structure with glycosylation patterns that hide many of the potential neutralizing antibody targets [60,61]. Several broad and potent mAbs have shown
promise in passive protection studies in nonhuman primates. PGT121, which targets a glycan-dependent site on the V3 loop of Env, elicited sterilizing immunity in macaques against SHIV infection at low doses of 1–5 mg/kg [62]. Other antibodies, including VRC01, 2F5, 2G12, b12, 4E10, 3BNC117, 10-1074, and others also elicited protection against SHIV challenge in non-human primates in combination or at higher doses [63–67].

Multiple approaches are being pursued to elicit bNAbs by immunization. One approach consists of designing immunogens that better mimic bNAb epitopes, for example, novel native-like Env trimeric proteins such as BG505 SOSIP.664 or bNAb epitope mimics on scaffolds [68–71]. Another approach is to use immunogens that bind germ-line precursors of bNAbs and thus stimulate different stages of the bNAb clonal lineage and allow for evolution of B-cells to production of bNAbs, known as B-cell lineage vaccines [72].

Another option for the delivery of bNAbs is gene transfer, which is sometimes referred to as vectored immunoprophylaxis. Adeno-associated virus (AAV) vectors are being used for this task to enable the direct expression of bNAbs. SIV modified immunoadhesins expressed via AAVs showed protection and long term neutralization in a non-human primate study [73]. AAVs have also been used to induce lifelong mAb production in humanized mice, affording protection against HIV [74]. Another immunoadhesin, CD4-Ig, was combined with a modified CCR5 binding peptide without inhibiting hydrophobic residues to enhance Env binding. This combination was expressed for up to 10 months in non-human primates and showed protection against SHIV challenge [75*]. One AAV delivering the neutralizing antibody PG9 is currently being tested in a phase I study (NCT01937455).

Vaccine strategies to induce bNAbs are in their early stages in terms of clinical development and a full review of strategies to induce bNAbs is beyond the scope of this review. Efforts to administer bNAbs passively are therefore being pursued in proof-of-concept studies to establish whether bNAbs protect against HIV-1 infection in humans. The first bNAb to begin human clinical trials is VCR-HIVMAB060-00-AB (VRC01), which targets the CD4 binding site. VRC01 neutralizes an extensive global panel of Env-pseudotyped viral strains in vitro [22,76,77], and passive transfer of VRC01 alone with two other bNAbs 10E8 and PG9 showed complete protection against SHIV challenge in rhesus macaques [67]. Thus far, VRC01 has been shown to be safe and well-tolerated, and to suppress viral replication transiently in HIV-1-infected individuals in the Phase 1 clinical trials VRC601 and VRC602 [78,79]. A phase 2b safety and efficacy study of VRC01 has recently begun and early phase clinical trials of other bNAbs are underway (NCT02256631; NCT02568215 (HVTN 703); NCT02599896 (VRC606)).

Vaccines that elicit effector memory T cells at mucosal sites of infection

The HIV-1 vaccine strategies discussed above aim to elicit humoral immune responses — V2-specific, poly-functional, and broadly neutralizing antibodies — as these responses may correlate with reduced risk of HIV-1 acquisition. Data from non-human primates also suggests that replicating viral vectors may elicit cellular immune responses that can prevent systemic HIV-1 infection by limiting the spread of mucosally acquired infection. For example, a rhesus cytomegalovirus (RhCMV) vector expressing SIV antigens elicited SIV-specific effector-memory T cells responses at mucosal sites of infection [80], prevented systemic, progressive dissemination of infection [81], and led to control of viremia and pathogenesis against SIVmac239 in rhesus macaques [82**]. Such an approach suggests that the RhCMV vector established a persistent HIV-1 effector T-cell pool that could rapidly control viral replication at the time of infection.

Several replicating vectors are being explored for their ability to induce persistent immune responses via continuous antigen stimulation, especially at mucosal sites of potential infection. The hypothesis is that these immune responses may inhibit viral infection prior to establishment of a viral reservoir and diversification of the infecting virus, thus containing or aborting early infection. In addition to modified attenuated human CMV vectors, which are not yet in clinical trials [82**], replicating adenoviruses (Ad) are also being explored, as they persist in mucosal sites [83]. For example, mucosal IgA titers following immunization with a replicating Ad-SIV vaccine in macaques correlated with improved control of viremia [84]. Clinical advancement of replicating Ad vectors is underway with replication-competent recombinant adenovirus serotype 4 (Ad4) HIV-1 vaccine regimens (Ad4-mgag and Ad4-EnvG150); a phase 1 clinical trial testing the oral and intranasal routes of administration is currently underway (NCT01989533). In addition, an oral replicating adenovirus serotype 26 (Ad26) vector expressing HIV-1 Env is also in Phase 1 testing (NCT02366013) [85]. Additionally, the vesicular stomatitis virus (VSV) vector has entered phase 1 clinical testing (NCT01438606) [86], and plans are in place to conduct a follow-up trial using a DNA prime-VSV vector boost (NCT01578889).

Conclusions

An HIV-1 vaccine has been a daunting challenge due to global HIV-1 diversity and the difficulties in inducing protective antibody responses and cellular immune responses. Here we discuss four current approaches to the development of a successful HIV-1 vaccine, each based on different immune correlates of protection. The first approach is to develop vaccines that elicit V2-specific antibody responses and that are well matched to
locally circulating strains. Examples of this approach include efforts to build on the ALVAC/AIDSVAX B/E vaccine regimens tested in RV144 with ongoing clinical trials in Thailand and Africa. The second approach is to develop vaccines to elicit diverse, polyfunctional antibody responses. Examples of this approach include efforts to test Ad26 vectors, mosaic immunogens, and trimetric gp140 protein regimens. The third approach is to elicit (or deliver) broadly neutralizing antibodies through immunization, gene delivery, or passive administration. The fourth strategy is to elicit effector memory T cells through the use of replicating vectors. As all four of these approaches move towards efficacy trials, the field will have an unprecedented opportunity to test prospectively which immune correlates are confirmed and afford protection against HIV-1 in humans.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


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