Novel strategies are being researched to discover vaccines to prevent and treat HIV-1. Non-efficacious preventative vaccine approaches include bivalent recombinant gp120 alone. HIV gene insertion into an Adenovirus 5 (Ad5) virus vector and the DNA prime/Ad5 boost vaccine regimen. However, the ALVAC-HIV prime/AIDSVAX B/E gp120 boost regimen showed 31.2% efficacy at 3.5 years, and is being investigated as clade C constructs with an additional boost. Likewise, although multiple therapeutic vaccines have failed in the past, in a non-placebo controlled trial, a Tat vaccine demonstrated immune cell restoration, reduction of immune activation, and reduced HIV-1 DNA viral load. Monoclonal antibodies for passive immunization or treatment show promise, with VRC01 entering advanced clinical trials.

Preventative HIV-1 vaccines

Initial HIV vaccines using recombinant envelope proteins

Following the traditional vaccine paradigm, the HIV-1 preventative vaccine field first developed over 20 different recombinant envelope proteins from various strains in the late 1980s to mid-1990s, hoping to induce neutralizing antibodies to HIV. The two recombinant gp120 vaccines tested in phase 3 were bivalent subtype B/B and bivalent subtype B/E, but neither proved efficacious [1,2].

What emerged from early immunogenicity studies was that although these vaccines induced both binding and neutralizing antibodies, the latter were often limited to the strain used in the vaccine [3]. This narrow neutralizing response is because of auto-reactivity and deletion of the precursor B cells that lead to the development of broadly reactive neutralizing antibodies [4]. Interestingly, post hoc analyses suggested that persons with high levels of blocking and binding antibodies may have had some protection from acquisition, a finding of minimal interest until the RV144 trial [5].

Adenovirus 5 (Ad5) vector HIV vaccine

The failure of the recombinant envelope vaccines shifted the focus to immune responses that would achieve cross-strain breadth. Emphasis was placed upon vaccines that induced CD8+ T-cell responses to HIV-1, in the hope that they would be directed at conserved regions of HIV and therefore be effective across different populations and clades. Pathogenesis studies revealed that the magnitude and breadth of the early CD8+ T-cells markedly influenced early viral control, so cytotoxic T-cell (CTL)-based vaccines were designed primarily to control post-infection viremia, but there were also hopes they could prevent HIV acquisition. The strategy to induce CTL responses to HIV proteins was to insert HIV genes into recombinant viral vectors and shuttle these genes into the Class I antigen-presenting pathway [6].

The first T-cell vaccine candidate to undergo clinical efficacy trials was a replication-defective recombinant Ad5 vector with HIV-1 clade B gag/pol/nef inserts. It had promising non-human primate data and exceptional human immunogenicity. Containing no envelope genes, this vaccine tested the concept of whether a CD8+ T-cell response would reduce post-acquisition viremia. It was given as three injections (0, 1, 6.5 months) in two phase 2b trials starting in 2004 (‘Step’) and 2007 (‘Phambili’). Later

Introduction

Despite early optimism about immunological approaches to prevent and treat human immunodeficiency virus (HIV), after more than three decades there are no licensed HIV preventative or therapeutic vaccines. This paper outlines the path toward novel vaccine designs that employ active and passive immunization strategies to prevent HIV acquisition, and the efforts toward a therapeutic vaccine (Figure 1).
in 2007, when futility was declared for the efficacy objective of Step, both Step and Phambili discontinued enrolment and vaccination, unblinding participants and continuing safety follow up.

Both trials revealed unexpected findings. The Step data, in men who have sex with men (MSM), showed that vaccine-recipients with pre-existing immunity to Ad5 and/or who were uncircumcised had an increased risk of HIV-1 acquisition which waned with time [7]. Phambili, conducted in heterosexual adults, showed no vaccine effect on HIV acquisition during blinded follow-up, but during the unblinded follow-up there was higher HIV-1 [8] phenomenon could not be attributed to circumcision status or baseline Ad5 sero-positivity [9]. The mechanism of increased HIV-1 acquisition has not been deciphered [10].

Step had further repercussions because it was found that the vaccine produced what were felt to be reasonable levels of CD8+ T-cell responses as well as long-standing immune responses recognizing clades B and C, with no effect on HIV-1 acquisition or viral load set-point. Post hoc analysis indicated that these CD8+ immune responses were directed at variable, not conserved, regions of the virus. Hence ‘immune T-cell breadth’, like neutralizing antibody breadth, was still an issue that needed to be solved [11].

DNA and Ad5 vector — another T-cell based approach

Shortly after Step was initiated, a strategy was developed to prime Ad5 with DNA. Incorporating vaccine strains from every major HIV-1 clade, it attempted to overcome the antibody and T-cell breadth problems. The DNA vaccine (0, 1, 2 months) contained a mixture of six plasmids expressing clade B gag, pol, nef and env proteins from clades A, B, and C, followed by a Ad5 vector boost (month 6) expressing a clade B gag-pol fusion protein and env glycoproteins from clades A, B, and C. The non-human primate model demonstrated protection from low-dose mucosal challenge [12]. The Phase 2A human clinical study, HVTN 204 demonstrated CD4+ T-cell responses to HIV-1 envelope, little neutralizing antibodies, but elicited binding antibodies to gp41 and HIV gp120 [13].

HVTN 505 evaluated the regimen in Ad5-seronegative circumcised MSM. Although increased rates of HIV acquisition were not seen, there was no efficacy and no reduction in viral load setpoint [14]. The reasons for failure are under speculation. Studies reveal that much of the gp41 antibodies elicited by vaccination were cross-reactive to an E. coli antigen which may have served as a
blocking antibody [15]. Recent studies point to viral escape mechanisms conferring neutralization resistance [16].

Pox-vector and protein vaccine combination

RV144 (2004–2009) proved that a vaccine approach could reduce HIV acquisition. It found 60.5% vaccine efficacy at 1 year and 31.2% vaccine efficacy at 3.5 years with a canarypox vector prime, ALVAC-HIV (vCP1521) expressing clade E env and clade B gag and pro (0, 1, 3, 6 months) followed by protein boosts with alum adjuvant, AIDSVAX® clades B/E gp120 (3, 6 months) [17**].

In preclinical testing, ALVAC-HIV had been less immunogenic than many other prototypes in human testing [18,19]. Moreover, the solo use of AIDSVAX® as well as a bivalent clade B gp120 had been ineffective in preventing HIV-1 acquisition [1,2]. That the combination of the immunogens would reduce HIV-1 acquisition among heterosexual Thai adults was astonishing.

None of the sera from RV144 recipients, even at peak levels of antibody response, neutralized a panel of 20 contemporaneous isolates of HIV-1 circulating in Thailand during the course of the trial. However, essentially all RV144 vaccine-recipients developed binding antibodies to gp120 [20**]. Post-vaccination sera tested against a linear peptide array derived from an HIV strain used in both the vector and gp120 exhibited a high binding pattern to peptides in the V1V2 region, especially the conserved amino acids on the V1V2 crown, residues 163–178. Vaccine-recipients with the highest magnitude of binding antibody titers were more likely to be protected than those with lower titers, with a reasonably linear association between vaccine efficacy and the peak concentration to the V1V2 scaffold. Epitope mapping of the antibodies to V1V2 indicated that much of the immune response was directed at a linear epitope interval including a lysine residue at amino acid 169 in the env V2 region. Antibodies from RV144 vaccine-recipients that bound to the K169 V2 region did not neutralize nor capture hard-to-neutralize (tier 2) subtype AE viruses but did bind to env on tier 2 AE virus-infected CD4+ T cells and mediate antibody-dependent cellular cytotoxicity (ADCC).

The arising hypothesis is that one mediator of the protective anti-HIV-1 antibody effector function induced by the RV144 vaccine was a non-neutralizing and most likely Fc receptor (FcR)-mediated action that included ADCC activity. Interestingly, V1V2 responses were not elicited by the DNA/Ad5 regimen (HVTN 505), even though these sequences were present in 2 of the 3 envelope vaccine constructs [21].

Genetic sieve analysis of viral isolates from HIV-infected participants revealed that isolates from vaccine-recipients were less likely to possess a lysine at K169 of the env V2 region than placebo-recipients, and vaccine efficacy was significantly higher against HIV-1 manifesting a lysine at K169 than against HIV-1 with a different residue at position 169, suggesting vaccine immune pressure at this region [22]. Thus, the immunological and virological data concur the V2 region of HIV-1 is a vulnerable point, and a target site of protective antibodies associated with vaccine efficacy of the RV144 regimen.

Building on RV144 (Figure 2)

In 2010, shortly after the announcement of the RV144 results, the pox protein public–private partnership (P5 Partnership) convened to develop the pox-vaccine regimen for sub-Saharan Africa. HVTN 097 evaluated the RV144 regimen in South Africa, a different setting to Thailand in terms of circulating HIV clade, predominant modes of transmission and average body mass index. Immunologic response in South Africans was comparable to, if not slightly better than, responses induced in Thailand [23].

P5 redesigned the ALVAC vector with a clade C env insert and constructed a bivalent clade C recombinant gp120. These vaccines are being tested in southern Africa with two different adjuvants, MF59® and AS01B, to try to improve responses and the regimen includes a 12 month boost to extend durability. Pending results from HVTN 100, a phase 1/2 study, ALVAC/gp120/MF59® is scheduled for evaluation in a pivotal efficacy trial in late 2016.

Other prime-boost approaches

Another approach for improving upon RV144 is to use more immunogenic vector platforms to improve B- and
T-cell priming, especially to envelope proteins. Investigations into an Ad35 vector platform showed tolerability and immunogenicity as a prime to Ad5 and as a boost to DNA immunization [24]. A leading second-generation vector under development is replication-incompetent Ad26 in combination with modified vaccinia Ankara (MVA) and recombinant protein. The vectors contain novel mosaic inserts designed to elicit cross-clade immunity [6]. Non-human primate studies using Ad26/MVA/trimeric gp140/ASO1B adjuvant demonstrated protection from mucosal challenge. Envelope-specific non-neutralizing binding antibodies are the main correlates of protection in these animals.

Neutralizing antibodies
The lack of broadly neutralizing antibodies elicited by the RV144-like regimen is an acknowledged deficiency in their immune profile. A concerted international effort is researching immunogens that will elicit broader neutralizing antibodies. The trimeric structure of HIV, defining masking epitopes and eliminating them from a potential vaccine immunogen, and using sequential heterologous isolates to ‘coax’ the immune system toward the development of broadly neutralizing antibodies are all under study [25].

Antibody-mediated prevention: monoclonal antibody approaches
Passive immunization is the administration of antibodies to prevent infection. Antibodies have been isolated which can neutralize a broad range of HIV strains in vitro, raising hopes for antibody-mediated prevention (AMP) of HIV. The first antibody to enter advanced human clinical trials is VRC01, discovered in an elite viral controller [26]. It is a human monoclonal antibody targeting the HIV-1 CD4 binding site. VRC01 demonstrated protection in animal studies, and has acceptable human safety [27*]. HVTN 703 will investigate the effectiveness of VRC01 and the level of neutralizing activity required in reducing HIV acquisition.

Therapeutic vaccines
Even if only partially effective, therapeutic vaccines may be valuable for HIV-infected individuals as treatment intensification on the immune system and viral reservoir when combined with highly active antiretroviral therapy (HAART), as a safeguard during suboptimal adherence, and/or as a cure adjunct. Vaccination at the time of primary infection or early in the course of infection has been proposed to limit reservoir establishment and promote viral eradication, analogous to the rationale for early HAART initiation. Unfortunately, in the past 20 years, almost fifty safety, immunogenicity and some efficacy therapeutic HIV vaccine trials have been evaluated, in acute, early and chronic HIV infection, and all with minimal success [28,29]. Newer approaches concentrate on antigen selection and vaccine delivery systems (Table 1).

<p>| Table 1 |</p>
<table>
<thead>
<tr>
<th>Current therapeutic HIV vaccine approaches</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactivated whole virus depleted of gp120</td>
</tr>
<tr>
<td>Single or multiple HIV antigens administered as DNA</td>
</tr>
<tr>
<td>Autologous dendritic cells</td>
</tr>
<tr>
<td>Viral vectors, for example, poxviruses (canarypox ALVAC-HIV, vCP1452, vCP1453, fowlpox, MVA), adenoviruses (Ad5)</td>
</tr>
</tbody>
</table>

Tat therapeutic HIV vaccine
The Italian National AIDS Center is developing a vaccine targeting the HIV-1 transactivator of transcription (Tat) protein, a key virulence factor which plays critical roles in HIV gene expression, replication, transmission and disease progression [30].

Tat-specific antibodies appear to be a possible key to prevent HIV acquisition and spread. Notably, anti-Tat antibodies are uncommon in natural infection [31,32] and, when present, correlate with the asymptomatic state and lower disease progression [33–35].

Phase 1 preventative and therapeutic studies demonstrated that Tat vaccination is safe and immunogenic [36–38]. A phase 2 study that was not blinded and did not have placebo controls suggest that in virologically-suppressed HAART-treated participants, Tat vaccination induced restoration of CD4+ and CD8+ T-cell numbers and functional central memory T-cell subsets, of B and natural killer (NK) cell number and a reduction of immune activation [39,40*]. Crucially, Tat immunization induced a statistically significant reduction of blood HIV-1 DNA load that persisted for up to three years post-vaccination. HIV-1 DNA decay was associated with anti-Tat antibodies and neutralization of Tat-mediated entry of oligomeric env in dendritic cells [39,40*]. The Phase 3 trials being developed would benefit from designs that incorporated placebo and double-blinding.

Therapeutic HIV antibodies
Broadly neutralizing antibodies offer not only prevention potential, but also treatment. In early-phase clinical trials, VRC01 and another human monoclonal antibody, 3BNC117, reduced viral load in HIV-1-infected individuals not on HAART [41,42*].

Conclusion
After numerous non-efficacious preventive and therapeutic vaccine candidates, pox/gp120 regimens, monoclonal antibodies, and Tat vaccines are amongst the latest approaches for exploration.

Conflict of interest statement
We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that
could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

**Funding source**

Funding source for this manuscript not applicable.

**References and recommended reading**

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

- of special interest
- of outstanding interest


This immune-correlates study of RV144 suggested that V1V2 antibodies were associated with protection against HIV-1 infection in vaccine recipients. Env-specific lgA antibodies was inversely correlated.


This study demonstrated that a manufactured human broadly neutralizing antibody is safe and could be evaluated further as a prevention modality.


This study is the first promising clinical trial of a therapeutic HIV vaccine.


42. Lynch RM, Boritz E, Coates EE, DeZure A, Madden P, Costner P, Enama ME, Plummer S, Holman L, Hendel CS et al.: Virologic effects of broadly neutralizing antibody VRC01 administration during chronic HIV-1 infection. Sci Transl Med 2015, 7:319ra206. This study demonstrated that a manufactured human broadly neutralizing antibody is safe and could be evaluated further as a treatment modality.