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New prospects for a preventive HIV-1 vaccine

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Abstract

The immune correlates of risk analysis and recent non-human primate (NHP) challenge studies have generated hypotheses that suggest HIV-1 envelope may be essential and, perhaps, sufficient to induce protective antibody responses against HIV-1 acquisition at the mucosal entry. New prime-boost mosaic and conserved-sequence, together with replicating vector immunisation strategies aiming at inducing immune responses or greater breadth, as well as the development of immunogens inducing broadly neutralising antibodies and mucosal responses, should be actively pursued and tested in humans. Whether the immune correlates of risk identified in RV144 can be extended to other vaccines, other populations, or different modes and intensity of transmission, and against increasing HIV-1 genetic diversity, remains to be demonstrated. Although NHP challenge studies may guide vaccine development, human efficacy trials remain key for answering the critical questions leading to the development of a global HIV-1 vaccine for licensure.

Keywords: HIV vaccine, HIV prevention, clinical trials, efficacy, immune correlates

Introduction

At the end of 2013, 35 million people were living with HIV worldwide, sub-Saharan Africa bearing the heaviest burden. While 70% of HIV cases are attributed to heterosexual transmission, the remaining 30% are attributed to a combination of men who have sex with men (MSM), mother-to child transmission and people who inject drugs (PWID) [1]. Worldwide, the number of people, including children, with new HIV-1 infections has fallen by 38% since 2001. This result can be attributed to aggressive HIV prevention measures including behavioural interventions, wider access to antiretroviral treatment (ART), male circumcision, treatment of sexually transmitted infections, harm reduction and prevention of mother-to-child transmission [2–4]. New prevention strategies are being developed including topical microbicides, pre-exposure prophylaxis (PrEP), and ART for prevention (TasP) [5–11]. While current strategies have led to a decrease in HIV prevalence and HIV-related mortality, they have not stopped HIV transmission. Several factors justify the development of a globally effective HIV-1 vaccine: the challenges associated with human behaviour and acting responsibly in sexual settings, legal barriers in some countries and limited access to ART. Only 25% of US citizens living with HIV have achieved an undetectable viral load [12]. Vaccines represent the most cost-effective public health intervention to counter infectious diseases. Vaccines against diseases such as smallpox, poliomyelitis and diphtheria have reduced the prevalence and incidence of associated illness to eradication and negligible numbers; therefore, providing irrefutable support for the effectiveness of a vaccine strategy to counter HIV transmission [13].

Several challenges hamper the development of a prophylactic HIV-1 vaccine. The virus has extraordinary diversity, even within an infected host. It integrates quickly into host DNA, establishing latent reservoirs inaccessible to treatment and the immune system [14]. The envelope spike is heavily glycosylated and the virus makes use of non-critical, decoy immunodominant epitopes [15]. The substantial replicative capacity of HIV-1 facilitates rapid and adaptive sequence evolution driven by point mutations and viral recombination creating an unprecedented capacity to evade immune responses [16,17]. Broadly neutralising antibodies (bNAb) developed in 20% of HIV-infected subjects are insufficient to limit HIV disease progression within these subjects [18]. Animal models, while helpful in guiding clinical development, remain imperfect in predicting the outcome of human trials. Our understanding of immune correlates of protection has improved but remains limited.

Multiple HIV-1 vaccine Phase I and II trials have been conducted worldwide (reviewed in [19]). Only one of six HIV-1 vaccine efficacy trials was effective in preventing HIV-1 acquisition. The Thai Phase II trial, RV144, was the first trial that showed that a vaccine against HIV is possible, with efficacy of 31% [20]. We review the findings of key clinical efficacy trials and animal studies and develop new perspectives that may lead an HIV-1 vaccine to licensure.

Lessons learned from human efficacy trials and animal challenge studies

Both antibody and cell-mediated immune responses are now thought to be important to counter HIV-1 [21] in both the systemic and mucosal compartments, the entry point for sexual transmission [22]. Among the promising vaccine approaches to achieve this objective, the prime-boost concept was developed by priming the immune system with a vaccine consisting either of a recombinant vector or DNA plasmids expressing HIV-1 proteins, and boosting with soluble HIV-1 proteins or with another vector expressing HIV-1 proteins [23,24]. Table 1 outlines the HIV-1 vaccine efficacy trials conducted so far.

Vax004

The first HIV-1 vaccine efficacy trial, Vax004, enrolled women at high risk for heterosexual transmission and MSM in the Netherlands and North America. The vaccine was composed of monomeric gp120 envelope subunits derived from GNE8 and MN HIV-1 subtype B isolates, formulated in alum, and aimed at inducing neutralising antibody (NAb) to block HIV-1 transmission. HIV incidence did not differ significantly between vaccine and placebo recipients nor did it affect HIV-1 disease progression [25,26]. High NAb levels against the easy-to-neutralise MN strain were, however, significantly inversely correlated with HIV-1 incidence while low levels against more-difficult-to-neutralise viruses were not, suggesting that level and breadth of elicited NAb were not sufficient for protection. Stratification of HIV-1 acquisition by gender showed that NAb

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Table 1. Completed human HIV-1 vaccine efficacy trials

Trial	Details	Population	Area	Outcome
Vax004	AIDSVAX B/B gp120 (MN and GNE8 subtype B) gp120 in alum	MSM* and women who engage in high-risk behaviour	USA and Europe	No efficacy
Vax003	AIDSVAX B/E gp120 (subtype B MN and CRF01_AE CM244) gp120 in alum	PWID†	Thailand	No efficacy
HVTN 502/Merck 023/Step trial	MRKAd5‡ HIV-1 Gag/Pol/Nef subtype B	High-risk population, MSM, heterosexual men and women	USA	No efficacy
HVTN 503/ Phambili trial	MRKAd5 HIV-1 Gag/Pol/Nef subtype B	Heterosexual men and women	Republic of South Africa	No efficacy; increased HIV infection observed in vaccinees
RV144	ALVAC-HIV§ (vCP1521) and AIDSVAX B/E (subtype B MN and CRF01_AE CM244) rgp120 in alum	Community risk	Thailand	31.2% efficacy against HIV acquisition at 42 months, 60% at 12 months. No effect on plasma viral load and CD4 count
HVTN 505	DNA Gag, Pol, and Nef from HIV-1 subtype B and Env from subtypes A, B, and C and rAd5 subtype B Gag-Pol and Env A, B, and C	Circumcised MSM and transgender individuals lacking infection with Ad5	USA	No efficacy

*Men who have sex with men; †people who inject drugs; ‡MRKAd5: recombinant replication-incompetent Ad5 vector; §ALVAC-HIV: recombinant canarypox vector

responses were higher in women than men, while behaviour and race had no significant effect [27]. Vaccination was associated with gp120-binding IgG in cervico-vaginal lavage, gingival secretions and gp120 plasma IgA [28]. An inverse correlation was found between antibody-dependent cell-mediated virus inhibition (ADCVI) activity and the rate of HIV acquisition among vaccinated individuals. Fc receptor IIIa genotype was associated with an increased rate of HIV-1 infection in low-risk, but not in high-risk vaccinees [29].

Vax003

Vax003, the only HIV-1 vaccine efficacy trial to target PWID, was conducted in Bangkok, Thailand. The vaccine consisted of bivalent gp120 envelope subunits, HIV-1 subtype B MN and CRF01_AE A244, in alum. The antibody levels prior to infection for gp120, A244 V2, A244 V3, blocking of A244 binding to CD4, and MN neutralisation were not significantly different between the HIV-infected and uninfected vaccine recipients and did not correlate with the rate of HIV infection [30].

The failure of these two trials redirected the focus of HIV-1 vaccine development into looking at the efficacy of vaccine-induced cell-mediated responses [19]. The rationale for T cell-based vaccines has been recently reviewed [31–33].

The Step trial

The Step trial (HVTN 502/Merck 023) tested a mixture of replication-defective recombinant adenovirus 5 vectors, MRKAd5, expressing HIV-1 subtype B *gag*, *pol* and *nef* genes, in MSM and women and men at high risk for HIV infection in the US [34]. The trial was halted after an interim analysis showing the vaccine did not confer protection against HIV-1 acquisition, or reduce post-infection plasma viral load despite detection of HIV-specific IFN-γ ELISPOT and intracellular cytokine-staining CD4+ and CD8+ T cell responses in a majority of vaccine recipients [35]. A *post hoc* multivariate analysis showed a greater risk for HIV-1 infection in uncircumcised men with pre-existing Ad5-neutralising antibodies [36]. The risk waned over time [37]. An additional study did not support sexual behaviour as a risk factor for increased HIV acquisition of uncircumcised individuals in the study who became HIV infected [38]. Ad5 antibodies were not correlated with increased risk for HIV acquisition in unvaccinated individuals [39]. Results from another study suggested that subjects infected during the Step trial seemed to have qualitative immune differences that increased their risk of HIV-1 infection independent of vaccination [40]. A sieve analysis showed evidence of vaccine-elicited immune pressure on the founder virus although no specific CD8+ cytotoxic T lymphocytes (CTL) recognising that epitope could be identified [41]. Despite evidence of anamnestic responses, the sieve effect was not well explained by available measures of T cell immunogenicity. Sequence divergence from the vaccine was not significantly associated with acute viral load [42]. Vaccinees with HLA alleles associated with HIV-1 control had a significantly lower mean viral load over time [43,44]. Intriguingly, non-HIV-specific IFN-γ ELISPOT magnitude was a significant direct correlate of risk (CoR) for HIV-1 infection in the vaccine group [45]. Interestingly, the most highly conserved epitopes were detected at a lower frequency, suggesting that stronger responses to conserved sequences may be as important as breadth for protection [46]. The outcome of the Step trial was recapitulated in an Indian rhesus macaque study where animals vaccinated with a regimen similar to that employed in the Step trial were not protected against an SIVsmE660 challenge [47]. Rhesus macaques chronically infected with a host-range mutant Ad5 (Ad5hr) and immunised with an rAd5 SIVmac239 gag/pol/nef vaccine were challenged with a series of escalating dose penile exposures to SIVmac251. Despite inducing CD8+ T cell responses in 70% of the monkeys, the vaccine did not protect vaccinated animals from penile SIV challenge [48].

The Phambili study

The Phambili study (HVTN 503), a parallel trial conducted in South Africa where the major circulating clade is HIV-1 subtype C, tested the MRKAd5 HIV-1 vaccine. After the results of the Step trial, the Phambili study was halted and treatment allocations unblinded. Only 801 of the 3000 participants had been enrolled in the study. Owing to this setback, statistical power of 80% to evaluate vaccine efficacy was not achieved. There was no evidence of vaccine efficacy, which did not differ by Ad5 antibody titre, gender, age, herpes simplex virus type 2 status or circumcision. There was no significant difference in viral load set-point although there was a trend for a lower viral load in

women. HSV-2 infection increased the risk of HIV-1 in men by five times, but not in women [49]. Variables such as early unblinding, untimely interruption of the study, and cessation of vaccinations may have introduced bias to the interpretation of results. An additional factor noted is that the demographics of the Step and Phambili studies were different; moreover, the men in the Phambili study were mostly heterosexual, while men in the Step study were MSM, which suggests that risk factors may also be different [50].

HVTN 505

HVTN 505 was an efficacy trial conducted in 21 sites in the United States. MSM or transgender women were enrolled to receive a vaccine regimen consisting of priming with a mixture of six DNA plasmids containing HIV-1 subtype B *gag*, *pol* and *nef*, and subtypes A, B and C *env* genes, and boosting with a replication-incompetent recombinant Ad5 vector expressing HIV-1 subtype B *gag-pol* and subtype A, B and C *env* genes.

In previous studies, the vaccine regimen was shown to be safe, well tolerated and induced polyfunctional CD4+ and CD8+ T cells, multi-clade anti-Env binding antibodies, and NAb against easy-to-neutralise Tier 1 viruses [51–54]. HVTN 505 was halted for futility, showing no efficacy and no statistically significant effect on viral load and a non-significant excess of HIV infection in the vaccinated group [55].

The vaccine regimen failed to protect NHP against SIVmac251 infection but conferred protection against an intrarectal challenge with SIVsmE660 in half the vaccinated monkeys. Additionally, a reduction in peak plasma virus RNA was observed in Mamu-A*01-positive monkeys, suggesting a role of cytotoxic T lymphocytes in the control of SIV replication. Low levels of neutralising antibodies as well as envelope-specific CD4+ T cell responses were also associated with protection [56].

Comment on recombinant adenovirus type 5-vectored trials

There has been much discussion about the results of these three efficacy trials [22,57]. The nature of futility analyses will hopefully prevent vaccine trials from progressing to the point where enhancement of infection is seen, and once unblinding is undertaken (and for ethical reasons this should be the default) it is difficult with *post hoc*, unblinded analyses to cleanly study questions related to HIV-1 infection. Adenovirus vectors differ in their use of surface receptors, patterns of induced RNA expression and immunogenicity [58], but responses to the major hexon protein may cross types and species [59,60].

A comparative study evaluated HIV susceptibility and phenotypes of human CD4+ T cells and found that Ad5-specific CD4+ T cells, naturally exposed or introduced via vaccination, are more susceptible to HIV *in vitro* than CMV-specific CD4+ T cells. Preferential losses or strong reductions of Ad5-specific T cell responses were also observed in HIV-infected individuals. Further analyses showed a prevalence of pro-inflammatory Th17-like, gut-mucosa homing phenotypes of Ad5-specific CD4+ T cells. Flow cytometry analysis of *in vitro* samples also showed increased preference for infecting both IL-17- and IL-2-producing Ad5-specific CD4+ T cells. This data suggests a possible mechanism for increased HIV infection rate in the vaccine recipients from the Step study. Additionally, the data suggest that HIV susceptibility testing of vector-specific CD4+ T cells from HIV vaccine candidates may be needed [61]. A recent analysis suggests that the proportion of cells, probably CD4+ T cells, producing IFN-γ without stimulation by exogenous antigen appears to carry information beyond T cell activation and baseline characteristics that predict risk of HIV-1 infection [45].

Although no increase in activated total or vector-specific mucosal CD4+ T lymphocytes was detected following Ad26 vaccination in humans [62], the assessment of gut CD4+ T cell activation should deserve particular attention in humans vaccinated with any vector, in particular adenovirus-based vectors. Taken together, the studies of recombinant Ad5 vectors raise an important, and more general question about the impact of vaccine vectors on HIV acquisition: the properties of viral vectors that confer adjuvant-like qualities may induce activation or display of homing markers and may render activated CD4+ T cells, and the host, more susceptible to HIV infection [63,64].

RV144

The vector prime and protein-boost concept was applied to RV144, a community-based Phase III efficacy trial that enrolled mostly heterosexual HIV-uninfected 18–30-year-old men and women at 'community risk' in Rayong and Chon Buri provinces in Thailand. The prime, ALVAC-HIV (vCP1521), was a canarypox vector expressing HIV-1 env (CRF01_AE 92TH023), gag, protease and the gp41 transmembrane anchoring region subtype B (LAI) genes. The boost, AIDSVAX B/E, was the bivalent monomeric gp120 soluble protein in alum, previously tested in Vax003. The trial participants received ALVAC-HIV or placebo at day 0, and then at 4, 12 and 24 weeks, and AIDSVAX B/E or placebo at weeks 12 and 24. The vaccine regimen was safe, well tolerated and judged adequate for public use [65].

HIV-1 incidence in the RV144 placebo group was 0.28 infections per 100 person-years, more than 10-fold lower than that observed in Vax003, Vax004, Step, Phambili or HVTN 505 [66]. In a pre-specified modified intention-to-treat analysis of 16,395 participants, which excluded seven individuals who were diagnosed with HIV at baseline, the vaccine efficacy was 31.2% at 42 months of follow-up after first vaccination. There were statistically non-significant differences in efficacy between men and women; however, the study was not adequately powered to analyse subgroups. No significant difference was seen in the mean plasma viral load and CD4+ T cell counts between the HIV-infected individuals of the vaccine and placebo groups [20,67]. *Post hoc* analytical data indicated vaccine efficacy was 44% at 18 months and 60% at 12 months after vaccination, indicating that vaccine efficacy was non-durable [68]. Post-infection CD4+ T cell count and HIV-1 RNA plasma set-point viral load were not different between recipients of vaccine or placebo [69]. A significant reduction in seminal fluid viral load was observed in vaccine recipients who became infected. As observed in previous Phase I and II trials, the vaccine regimen induced binding antibody against HIV-1 Env immunogens and p24 Gag, CD4+ proliferative responses (63%), some CD8+ T cell responses (24%), NAb against T cell line-adapted viruses of subtype B and CRF01_AE (96% and 71%, respectively), and antibody-dependent cell-mediated cytotoxicity (ADCC) [20,70,71]. Predominantly CD4+ T cell mediated, IFN-γ ELISPOT-positive responses were detected in 41% of the vaccinees and targeted the V2 region, which includes the α4β7 integrin-binding motif [72–74]. A majority of vaccine subjects (97%) had antibody responses against cyclic V2 peptide at 2 weeks post immunisation, declining to 19% at 28 weeks after last injection. Antibody responses targeted the mid-region of the V2 loop that contains conserved epitopes and has the amino acid sequence KQKVHALFYKLDIVPI (HXB2 numbering sequence 169–184) [75]. Intracellular cytokine staining confirmed that Env responses predominated (19 of 30; 63% of vaccine recipients) and were

mediated by polyfunctional effector memory CD4+ T cells, with the majority of responders producing both IL-2 and IFN-γ (63%). HIV Env antibody titres were higher in subjects with IL-2 compared with those without IL-2-secreting HIV Env-specific effector memory T cells. Proliferation assays revealed that HIV antigen-specific T cells were CD4+, with the majority (80%) expressing CD107a, a functional marker of natural killer cell activity [76]. Although the detection of neutralising activity against Tier 1 viruses was detected in both Vax003 and RV144, the Tier 1 NAb titres were higher after the RV144 regimen compared to two gp120 protein administrations alone, confirming a priming effect for ALVAC-HIV [77].

RV144 and immune correlates of risk

The definition of correlates of protection (CoP) was recently redefined to include mechanistic and non-mechanistic CoP. Correlates of risk (CoR) are the statistically relevant responses that may be a CoP, or indicative of susceptibility to a pathogen or genetic susceptibility to infection [78–81]. Immune CoP in HIV-1 and NHP vaccine studies, and more specifically in RV144, were recently reviewed [22,66].

The efficacy observed in RV144 led to a case–control study being conducted to determine the antibody and cellular immune CoR for HIV acquisition [82]. After a careful stepwise selection process, six primary assay variables were selected: CD4+ T cell responses, V1V2 binding antibodies, neutralising antibodies, ADCC, avidity of IgG antibodies for Env, and IgA antibodies binding to Env. Cryopreserved blood samples were analysed from individuals who became infected after vaccination (case) and on non-infected vaccinees (controls).

Plasma IgG antibodies to scaffolded V1V2 caseA2 (HIV-1 subtype B) envelope proteins correlated inversely with risk of infection, while plasma IgA-envelope binding correlated with risk. Neither low levels of V1V2 antibodies nor high levels of Env-specific IgA antibodies in vaccinees or those in the placebo group correlated with increased rates of infection, ruling out the possibility of vaccine-induced enhancement of risk of infection [82]. In vaccinated subjects with low levels of plasma Env-specific IgA, an inverse correlation with infection was observed between IgG avidity, ADCC, neutralising antibodies, and Env-specific CD4+ T cells. RV144 antibodies to subtype A, C and CRF01_AE gp70 V1V2 scaffold proteins also correlated inversely with risk [83], suggesting that the RV144 regimen might protect against heterosexual transmission of HIV strains heterologous (A and C) to the vaccine components. Two weeks after last vaccination, 97% of RV144 studied plasma samples from vaccine recipients contained antibodies to V2 region synthetic peptides, falling to 19% at 48 weeks, suggesting that waning vaccine efficacy may be correlated with waning V2 antibody response. Interestingly, gp70 V1V2 antibodies were lower in HVTN 505 compared to RV144 [55].

The antibody response to V3 CRF01_AE and neutralising antibodies also inversely correlated with the risk of HIV infection in vaccine recipients with lower levels of Env-specific plasma IgA. In Vax003 and Vax004 (no protection), serum IgG responses targeted the same epitopes as in RV144 with the exception of an additional C1 reactivity in Vax003 and infrequent V2 reactivity in Vax004. Moreover, IgG to linear epitopes in the V2 and V3 regions of gp120 correlate with reduced risk in RV144 [84].

A sieve analysis identified two vaccine-associated genetic signatures in V2 corresponding to sites 169 and 181, further supporting the hypothesis that vaccination-induced immune responses directed against the V2 loop were associated with protection [85]. Monoclonal antibodies from RV144 vaccine recipients bind the V2 K169 residue, providing additional evidence that vaccine-induced antibodies correspond to the observed sieve effect. These V2-specific antibodies can mediate ADCC, neutralisation and low-level virus capture [86,87]. These findings generate the hypothesis that V2 IgG plays a role in protection against HIV-1 acquisition but do not distinguish between mechanistic or non-mechanistic mechanisms of protection [80].

In previous clinical studies, monomeric gp120 induced high levels of Env-specific IgG4 antibodies [88] while ALVAC (vCP1452) prime and gp120 MN in alum boost elicited lower IgG4 relative to IgG1 and IgG3 antibodies [89]. Antigen-specific IgG3 antibodies are associated with a beneficial effect against several pathogens [90–92]. Conversely, IgG4 has been associated with progression to AIDS [93]. IgG3 can fix the complement and has a high affinity for Fc R [94]. In RV144, Env IgG3 was correlated with decreased risk of HIV infection, a response that declined rapidly compared to overall IgG responses [95]. A recent comparison of RV144 and Vax003 showed that Env-specific IgG3 and V1/V2 IgG3 response rates were higher in recipients of the RV144 vaccine compared to Vax003 vaccinees and conversely that IgG4 were considerably lower in RV144. V1/V2 IgG3 responses and IgG3 responses specific for V1/V2 169K correlated with decreased risk of HIV-1 infection after IgA adjustment [95]. Chung *et al*. recently showed that the RV144 regimen elicited highly coordinated Fc-mediated effector responses, with the selective induction of highly functional IgG3 antibodies. By contrast, Vax003 elicited monofunctional antibody responses influenced by IgG4 selection. Moreover, only RV144 induced IgG1 and IgG3 antibodies that targeted the crown of the HIV envelope V2 loop, although with low coverage of breakthrough viral sequences [96]. ALVAC priming, due to its unique pro-inflammatory cytokine and chemokine response following vaccination in rhesus monkeys and infection in human PBMC, might shift the IgG subclass response to IgG3 in humans after vector prime and envelope protein boost compared with envelope vaccination alone [97]. The contribution of Fc–FcγR interaction-mediated functions through mechanisms such as ADCC, ADCVI, and antibody-dependent cell phagocytosis (ADCP) antibodies remains to be explored [98,99]. A recent *post hoc* analysis of RV144 showed a positive association between the Fc RIIC polymorphism and vaccine efficacy, emphasising the role of host genetics in predicting vaccine efficacy [100].

The RV144 direct correlation of plasma IgA with risk of infection is puzzling [101]. It has been suggested that plasma IgA may block IgG activity involving ADCC and phagocytosis [102–104]. In RV144, IgA antibodies elicited by RV144 block C1 region-specific IgG-mediated ADCC [105]. Monoclonal antibodies from RV144 vaccine recipients appeared to bind to a region of V2 that partially overlaps the binding of the bNAb CH01 and PG9. Although not broadly neutralising, these V2-specific antibodies mediate ADCC. Further mapping of ADCC responses from RV144 volunteers shows that a significant portion of the immune responses is directed at the first constant region of gp120 Env (C1) [106]. These C1-directed antibodies are able to block the binding of the A32mAb, which recognises a conformational epitope that includes the C1 domain. The C1-specific antibodies appear to act synergistically with anti-V2 antibodies, possibly by inducing conformational changes that improve the exposure of V2 to binding [107]. IgA purified from serum of HIV-uninfected RV144 vaccinees was able to efficiently opsonise viral particles in the absence of significant aggregation, reflective of monomeric IgA. In contrast, dimeric IgA monoclonal

antibodies (mAbs) formed stable viral aggregates, suggesting aggregation as a potential protection mechanism at the mucosal portals of viral entry [108]. Table 2 outlines the immune CoR identified in clinical efficacy trials.

Innovative approaches to HIV-1 vaccine development

Antibody-based strategies

Capitalising on the RV144 results and correlates of risk analysis

Advanced development of the pox vector prime and protein-boost strategy is proceeding towards efficacy trials in Southern Africa (heterosexual transmission), Thailand (MSM), and China (MSM) [57]. This strategy aims at inducing both non-neutralising (nNAbs) and neutralising (NAbs) antibodies. Designing vaccines to elicit production and concentration of antibodies at mucosal frontlines could aid in the development of an effective vaccine to protect women and MSM against HIV-1 [109]. The role and plausible mechanisms of action of nNAbs in protection against HIV acquisition have recently been reviewed [110,111]. Data from three NHP studies [112–114] suggest that Env is a necessary component for successful protection from SIV acquisition. Mechanisms of protection against acquisition may include inhibition of transcytosis [99,115–120], possible hindrance of HIV mobility by trapping of viruses linked to IgG and IgA within mucin layers outside the cervico-vaginal epithelium [121,122], viral capture by sIgA and IgG, viral aggregation [108], ADCC and ADCVI [117,118,123–125], and ADCP [126]. In Vax004, ADCVI activity was associated with lower infection rates [127]. The inhibition of virus replication in mucosal tissues *ex vivo* along with HIV-specific nNAb in mucosal secretions is currently being explored in RV144 follow-up studies [128–130].

Antigen design aiming at better-presenting conformational epitopes that induce nNAbs is explored. The antigenicity of the A244 gp120 (used on RV144) C1 region and the V2 conformational epitopes could be enhanced by the deletion of 11 N terminus amino acids of gp120 (Δ11). Conformational V1/V2 mAbs gave significantly higher levels of blocking of plasma IgG from A244 Δ11 gp120 immunised animals than IgG from animals immunised with unmodified A244 gp120 [131].

Inducing broadly neutralising antibodies

The challenge for epitope-based vaccine design is that only broadly conserved and exposed epitopes are suitable for vaccine targeting, but these epitopes, in their natural context, tend to elicit poor antibody responses. Perhaps the most difficult step is to design, engineer and produce a stable envelope immunogen that mimics the antigenic profile of the functional envelope spike [132].

Few engineered trimeric envelopes have been able to induce bNAb in animals [133]. Removal of individual glycans proximal to CD4-binding region impairs viral infectivity and results in enhanced capability to induce neutralising activity in mice [134]. The elimination of the glycosylation site near the gp41 loop resulted in enhanced immunogenicity, but immunisation of monkeys with this protein and two others derived from patients with bNAb was not more immunogenic than with one [135]. A stable gp140 trimeric envelope induced bNAb against Tier 1 and Tier 2 viruses with titres substantially higher than those elicited by the corresponding gp120 monomers [136]. The use of multivalent mixtures of natural HIV-1 subtype C envelope immunogens elicited a greater magnitude of NAbs against a panel of Tier 1 viruses than any single clade C trimer alone, but not against Tier 2 viruses [137]. A combination of mosaic envelopes tested in macaques increased the magnitude of NAbs but not the breadth of the response [138]. So far, no trimeric envelope induces bNAb in humans [139].

Another approach, called B cell lineage vaccine design, aims to engage the naïve B cell repertoire residing in bone marrow and secondary lymphoid tissue. Specifically, one or more clonally related bNAb must be isolated and, using next generation sequencing, an antibody lineage constructed through inference that links the mutated bNAb-producing cell to its naïve, germline ancestor. Recombinant antibody technology would express members of that bNAb lineage in order to select HIV-1 envelope constructs that optimally bind them. Those envelope constructs would be used as immunogens in a prime-boost to engage the naïve B cells *in vivo* and iteratively stimulate B cell 'evolution'

Table 2. Immune correlates of risk identified in HIV-1 vaccine efficacy trials

Trial	Details
RV144	Plasma IgG binding antibody to gp70V1V2 scaffold proteins (subtypes B, A, C and CRF01_AE) inversely correlated with risk of infection
	Plasma IgA-envelope binding antibodies correlated with risk
	In vaccine recipients with low plasma IgA antibodies, an inverse correlation was observed between rate of infection and Env-specific CD4+ T cells, ADCC, neutralising antibodies, and Env IgG avidity
	Sieve analysis showed two positions in V2 (169 and 181), which substantiates the hypothesis that protection resulted from vaccine-induced responses against V2 loop
	Positive association between the FcγRIIC polymorphism and vaccine efficacy
	Env IgG3 correlated with decreased risk of HIV infection
Vax004	ADCVI inverse correlated with rate of HIV acquisition
	High levels of neutralising antibodies to MN inversely correlated with HIV incidence
	Fcγ receptor IIIa genotype was associated with an increased rate of HIV-1 infection in low-risk, but not in high-risk vaccinees
Vax003	No correlates identified
Step trial (HVTN 502)	Presence of HLA alleles and overall T-cell breadth and magnitude of the immune response significantly correlated with lower mean viral load in infected vaccinees suggesting the implication of CD8+ cytotoxic T lymphocytes
	Non-HIV-specific ELISPOT magnitude was a significant direct CoR for HIV-1 infection in vaccinees
Vax003	Analysis ongoing

CoR: correlate of risk

until bNAb-producing cells are elicited [140]. A recent study determines the viral and antibody evolution leading to induction of a lineage of HIV-1 broadly neutralising antibodies, and provides insights into strategies to elicit similar antibodies by vaccination [141].

Vector-based delivery of broadly neutralising antibodies

Whether bNAb will effectively confer protection against HIV acquisition in humans remains a key question. An alternative to inducing bNAb by vaccination with immunogens is to deliver these bnMAb with viral vectors administered intramuscularly such as an adeno-associated virus (AAV) gene transfer vector expressing antibodies or antibody-like immunoadhesins with predetermined SIV specificity. SIV-specific molecules are endogenously synthesised in myofibres and passively distributed systemically. Long-lasting neutralising activity in serum was generated in monkeys and conferred complete protection against intravenous challenge with virulent SIVmac316 [142,143]. Similarly, full protection against intravenous HIV-1 challenge was obtained in humanised mice which received an AAV vector carrying full-length b12 antibody administered intramuscularly, while those receiving AAV expressing 2G12, 4E10 and 2F5 were partially protected [144]. Moreover, humanised mice receiving AAV vector carrying VRC07 were protected against repeated vaginal challenge with diverse HIV-1 strains [145]. An AAV vector carrying PG9 is now tested in a Phase I trial in Europe (www.iavi.org).

Cell-mediated-based strategies

Other avenues being explored aim to increase breadth of the CD4+ and CD8+ T cell immune responses and tackle HIV-1 genetic diversity [32,146,147]. Breadth and magnitude of T cell responses correlated with control of set-point viral loads in macaques vaccinated with vectors expressing core SIV proteins (no envelope) and challenged with SIV [148]. This increased breadth and depth of epitope recognition may contribute both to protection against infection and to the control of variant viruses that emerge as they mutate away from recognition by cytotoxic T lymphocytes [149].

Conserved sequences

A recent study of the contribution of the lower replication capacity of the transmitted/founder virus and an associated induction of a broad primary HIV-specific T cell response, which was not undermined by rapid epitope escape, to long-term viral control in HIV-1 infection underscores the importance of the earliest CD8+ T cell response that targets regions of the virus proteome that cannot mutate without a high fitness cost. This further emphasises the need for vaccines to elicit a breadth of T cell responses to conserved viral epitopes [150].

Immunogens, including HIV-1 conserved sequences, provide an effective strategy to broaden responses by causing reaction to critical viral elements for which few escape pathways exist [151–154]. Priming with the conserved element vaccine followed by boost with the complete immunogen induces broad cellular and humoral immunity focused on the conserved regions of the virus [155]. Conserved sequences and immune responses have been characterised in animals [156,157] and have conferred partial protection against SIVmac251in macaques [158]. In humans, a combination of DNA, ChAd63 and MVA vectors that was found safe [159] and immunogenic, induced high levels of effector T cells that recognised virus-infected autologous CD4+ cells. *In vitro* inhibition of HIV-1 replication was mediated by both Gag- and Pol-specific effector CD8+ T cells that targeted epitopes that were subdominant in natural infection [160].

Mosaic immunogens

Polyvalent mosaic immunogens derived by recombination of natural strains of HIV-1 are designed to induce cellular immune responses that recognise genetically diverse circulating virus isolates. Mosaic HIV-1 antigens expressed by recombinant Ad26 vectors markedly augmented both the breadth and depth of similar magnitude of antigen-specific T lymphocyte responses as compared with consensus or natural sequence HIV-1 antigens in rhesus monkeys [161]. Ad26/MVA and Ad26/Ad35 vector-based vaccines expressing HIV-1 mosaic Env, Gag and Pol significantly decreased risk of acquisition following repetitive, intrarectal SHIV-SF162P3 challenges. Protection correlated with vaccine-elicited binding, neutralising and functional non-neutralising antibodies, suggesting that the coordinated activity of multiple antibody functions may contribute to protection against difficult-to-neutralise viruses. However, the vaccine regimens had only a modest effect on viral set-point after challenge [113]. In contrast, similar vector regimens expressing SIVsmE543 antigens afforded >2 $\log_{10}$ reductions of set-point viral loads following heterologous SIVmac251 challenges [112]. Ad26 prime and MVA boost mosaic vectors should soon enter human clinical trials.

Replicating vectors

Mimicking the benefits of live-attenuated vaccines, the replicating vector approach was developed from the concept that persistent antigen exposure could confer immune control over viral exposure [162,163]. Live-attenuated SIV vaccine-mediated protection against SIVmac239 challenge strongly correlated with the magnitude and function of SIV-specific effector T cells in the lymph node but not in the blood or with other immune parameters. The maintenance of lymphoid tissue-based, effector-differentiated, SIV-specific T cells that intercept and suppress early wild-type SIV amplification and can control and perhaps clear infection, provides a rationale for the development of persistent vectors such as cytomegalovirus that can elicit and maintain such response [164]. A rhesus cytomegalovirus (RhCMV)-based vaccine expressing SIV proteins induced and maintained a high frequency of SIV-specific CD4+ and CD8+ T cell effector memory (TEM) responses at extra-lymphoid sites without measurable antibody responses to SIV. While the vaccine did not protect against infection, half of the vaccinated monkeys showed a stringent control of intrarectally administered SIVmac239 for more than a year. The outcome of challenge was predicted by peak SIV-specific CD8+ TEM frequencies in peripheral blood before the challenge [165,166]. However, it remains unclear why only 50% of the vaccinated animals were protected. RhCMV-SIV vectors elicit SIV-specific CD8+ T cells that recognise unusual, diverse and highly promiscuous epitopes, including dominant responses to epitopes restricted by MHC-II molecules and modulated by specific genes, suggesting that CMV vectors can be genetically programmed to achieve distinct patterns of CD8+ T cell epitope recognition [167].

Out-of-the-box approach

Prior infection of rhesus macaques with an attenuated SHIV conferred protection against vaginal SIV challenge associated with SIV-specific CTL in cervical vaginal tissues [168], suggesting that a modest vaccine-induced CD8+ T cell response in the context of immune suppression of T cell activation may protect against vaginal HIV-1 transmission [169]. Intriguingly, oral immunisation of macaques with inactivated SIVmac239 induced immune tolerance and elicited CD8+ regulatory T cells (Tregs), completely protecting a majority of animals without inducing

SIV-specific antibodies or CTL [170].

Frequency, magnitude and duration of responses

The persistence and boosting of HIV vaccine-induced effector and central memory T cell differentiation as well as of humoral immune responses in the mucosal compartments after a long interval after primary vaccination has not been studied systematically. Long-term maintenance of the memory T cell response is the hallmark of immune protection and, hence, constitutes one of the most important objectives of vaccine-development strategies [171]. Clinical trials show that HIV-1 vaccine-induced immune responses are of modest magnitude and short duration [19]. Several strategies are proposed to remedy these issues. Recently, the RV305 trial vaccinated RV144 vaccine recipients 6–8 years after the original series. The ALVAC-HIV/AIDSVAX B/E combination induced antibody titres higher than the 2-weeks post fourth RV144 vaccination, suggesting an anamnestic response and long-term memory despite the interval between vaccinations [172].

The use of potent adjuvants may also augment and shape antigen-specific antibody responses and contribute to antigen dose sparing. Several adjuvants have been tested in NHP and humans [173] showing a significant benefit of HIV envelope proteins formulated with either MF59 [174] or AS01 [175,176]. In macaques vaccinated with ALVAC-SIV and SIV gp120 in alum or MF59, alum protected macaques from SIVmac251 acquisition while MF59 did not, despite its ability to elicit higher systemic T cell and antibody responses. MF59 altered homing of antibody-producing cells and increased frequency of CXCR3 plasmablasts in blood that positively correlated with anti-envelope IgA serum levels and phagocytosis. Alum, in contrast, increased the frequency of plasmablasts expressing the mucosal integrin a4b7, which positively correlated with IgA responses to cyclic V2 in rectal mucosa. In the alum group, mucosal IgG to cyclic V2 correlated with lower risk of SIVmac251 acquisition. However, mucosal IgG to linear and cyclic V2 correlated with an increased risk of SIVmac251 acquisition in the MF59 group [177]. The formulation of HIV-1 gp120 with L(MPLA) and alum induced significantly higher levels of neutralising antibodies and T cell lymphoproliferation compared to alum, MF59 or MPLA alone [178]. High titres of gp70 V1V2 caseA2 antibodies as early as post second protein administration were elicited. The frequency of antibody response to gp70 V1V2 caseA2 was 100% at 2 weeks post second vaccination and 10 months after the fourth vaccination in the L(MPLA) adjuvant + alum group, while 85% and 100%, respectively, in the alum group. These antibody titres were five- to 10-fold higher than those observed in the group that received the antigen formulated with alum, and were detected at high levels 40 weeks post fourth vaccination and were higher than those observed in RV144. Moreover, antibodies were cross-reactive with gp70 scaffolds for CRF01_AE and subtype C [179]. Formulation of antigens with solid nanoparticles may prolong the duration of antibody responses by increasing antigen deposition/retention locally in the tissue that drives B cell responses. Dendritic cell antigen presentation [180] and development of CD4+ Tfh cells [181] would therefore be enhanced, which would provide critical cytokines and signals required to initiate somatic hypermutation and affinity maturation for effective B cell memory [182].

Modes and routes of administration

The vast majority of HIV-1 vaccine administrations are made by intramuscular injections. DNA constructs induce a better immune response via the intradermal rather than intramuscular or subcutaneous route [183,184]. Electroporation has also been tested with success [185,186]. Immunisation that targets iliac lymph node in macaques with SIV proteins elicited the most consistent mucosal antibody responses in the rectum, vagina, urine, seminal fluid, and blood [187]. Subcutaneous inguinal administration of ALVAC-HIV (vCP205) in humans induced qualitative or quantitative compartmentalisation of immune responses between blood and gut mucosa [188]. Other routes that aim to induce gut and cervico-vaginal mucosal responses remain poorly explored with mixed results including rectal [189–191], vaginal [192,193], oral [194,195], intra-ileal [196], nasal [197,198], aerosol [199], and combined systemic and mucosal [200,201] administrations. Co-delivery of DNA and protein HIV-1 vaccines has been tested in mice and NHP and appears to have an overlap of the prime-boost effect, achieving better immune responses than each component alone [202–204].

Conclusion

Put in perspective, the RV144 CoR analysis and recent NHP challenge studies have generated hypotheses suggesting that Env is essential and perhaps sufficient to induce protective antibody responses against HIV-1 acquisition at the mucosal entry. New prime-boost mosaic and conserved sequences, replicating vector immunisation strategies aimed at inducing immune responses of greater breadth and depth, as well as the development of immunogens inducing broadly neutralising antibodies and mucosal responses are exciting new approaches that will soon enter early-phase testing in humans. Whether the immune CoR identified in RV144 can be extended to other populations with different modes and intensity of transmission and against increasing HIV-1 genetic diversity remains to be demonstrated. Although NHP challenge studies may guide vaccine development, human efficacy trials remain key to answer the critical questions leading to the development of a global HIV-1 vaccine for licensure [205].

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