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Novel HIV vaccine strategies: overview and perspective

Yehuda Z. Cohen and Raphael Dolin

Abstract: A human immunodeficiency virus (HIV) vaccine remains a central component in the quest to control the worldwide epidemic. To examine the status of the development of HIV vaccines, we review the results of the efficacy trials carried out to date and the immunologic principles that guided them. Four vaccine concepts have been evaluated in HIV-1 vaccine efficacy trials, and the results of these trials have provided significant information for future vaccine development. While one of these trials demonstrated that a safe and effective HIV vaccine is possible, many questions remain regarding the basis for the observed protection and the most efficient way to stimulate it. Novel HIV vaccine strategies including induction of highly potent broadly neutralizing antibodies, use of novel homologous and heterologous vector systems, and vectored immunoprophylaxis seek to expand and build upon the knowledge gained from these trials.

Keyword: HIV vaccine, HIV vaccine clinical trials, HIV vaccine review, AIDS VAX, Step, RV144, HVTN 505

Introduction

Despite almost 30 years of intensive research, a human immunodeficiency virus (HIV) vaccine remains elusive. Effective vaccines generally stimulate protective immunity similar to that which occurs during natural infection. However, naturally acquired immunity against HIV infection may not exist, which presents an unprecedented challenge for vaccine development. The mechanism by which an HIV vaccine might confer protection therefore remains uncertain, and an effective vaccine may require induction of an immune response that is significantly different from that seen during natural infection [Johnston and Fauci, 2011]. The extreme diversity of HIV presents another challenge to vaccine design. Considering its relatively recent origin, the diversity of HIV, and HIV-1 in particular, is extraordinary. Within the main HIV-1 subgroup, Group M, there are nine clades as well as dozens of recombinant forms, and clades can vary up to 42% at the amino acid level [Hemelaar, 2012]. A vaccine immunogen derived from a particular clade may therefore be ineffective against other clades, posing a significant obstacle to the creation of a global HIV vaccine.

In this review, we examine the current status of HIV vaccine research by reviewing the results of

candidate HIV vaccine efficacy trials and the immunologic principles that guided them. We also review novel approaches that seek to build upon the strategies used in those trials. Four vaccine concepts have been evaluated in efficacy trials to date. The VAX004 and VAX003 trials evaluated the first concept, a protein subunit vaccine. The second concept, a recombinant adenovirus vector, was evaluated in the Step and HIV Vaccine Trials Network (HVTN) 503/Phambili trials. The third concept, a canarypox vector prime followed by a protein subunit boost, was evaluated in the RV144 trial. The fourth concept, a DNA prime followed by a recombinant adenovirus vector boost, was recently evaluated in the HVTN 505 trial. These trials are presented in chronological order, and their results are summarized in Table 1. We conclude this review with a discussion of additional novel strategies that have the potential to significantly advance HIV vaccine development.

Protein subunit vaccines: the VAX trials and the search for neutralizing antibodies

The first HIV vaccine candidates to enter clinical trials were protein subunit vaccines. Prior to the development of protein subunit vaccines, both

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Table 1. HIV vaccine efficacy trials.

Efficacy trial	Vaccine	Population	N	Efficacy	Other significant results	Immune response	Immune correlates of risk
VAX004	AIDSVAX B/B (rgp120 immunogens)	Primarily high-risk MSM	5403	None [Flynn <i>et al.</i> 2005]	N/A	Weak nAb response [Gilbert <i>et al.</i> 2010]	N/A
VAX003	AIDSVAX B/E (rgp120 immunogens)	Injection drug users	2546	None [Pitisuttithum <i>et al.</i> 2006]	N/A	Weak nAb response [Montefiori <i>et al.</i> 2012]	N/A
Step	MRKAd5 HIV-1 (rAd5 vector expressing Gag, Pol, and Nef)	Primarily high-risk MSM	3000	None; trial halted after meeting prespecified fertility boundaries [Buchbinder <i>et al.</i> 2008]	Significantly increased risk of HIV infection in men who were both Ad5-seropositive and uncircumcised, which waned with time since vaccination [Duerr <i>et al.</i> 2012]	CD8 ⁺ T-cell response detected in the majority of vaccinees, although weak and of narrow breadth [McElrath <i>et al.</i> 2008]	N/A
HVTN 503/ Phambili	Same as Step	Primarily heterosexuals	801	None; enrollment halted after lack of efficacy seen in Step [Gray <i>et al.</i> 2011b]	N/A	Similar to Step [Gray <i>et al.</i> 2011b]	N/A
RV144	ALVAC (canarypox vector expressing Env, Gag, and Pol) prime followed by AIDSVAX B/E boost	Primarily low-risk heterosexuals	16,402	31.2% overall efficacy for prevention of HIV-1 infection in the modified intention-to-treat analysis [Rerks-Ngarm <i>et al.</i> 2009]; no subsequent effect on viremia or CD4 count in vaccinees who were infected [Rerks-Ngarm <i>et al.</i> 2012]	68% efficacy for low or medium risk participants, no efficacy in the high-risk group; efficacy was highest over the first 12 months and then fell rapidly [Robb <i>et al.</i> 2012]	Weak nAb response [Montefiori <i>et al.</i> 2012]. Moderate CD8 ⁺ and CD4 ⁺ T-cell response; the CD4 ⁺ T-cell response was directed against the V2 region of Env [de Souza <i>et al.</i> 2012]	Binding of IgG to the V1 and V2 regions of Env correlated with protection; protection was mitigated by the presence of plasma IgA directed against Env [Haynes <i>et al.</i> 2012a]
HVTN 505	VRC-HIVDNA016-00-VP (DNA expressing Gag, Pol, Nef, and Env) prime followed by VRC-HIVADV014-00-VP (rAd5 expressing Gag, Pol, and Env) boost	High-risk MSM	2504	None; trial halted after meeting prespecified fertility boundaries	Awaiting final trial results	Awaiting final trial results	N/A

HIV, human immunodeficiency virus; HVTN, HIV Vaccine Trials Network; IgG, immunoglobulin G; MSM, men who have sex with men; nAb, neutralizing antibody; rgp, recombinant glycoprotein; rAd5, recombinant adenovirus serotype 5.

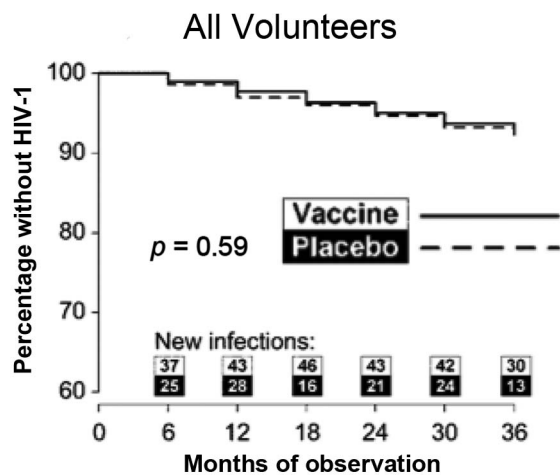


Figure 1. Kaplan–Meier curve from VAX004 showing time to human immunodeficiency virus type 1 (HIV-1) infection. (Reproduced from Flynn *et al.* [2005] with permission from Oxford University Press.)

attenuated and inactivated vaccines had been tested in nonhuman primates (NHPs), but neither concept advanced to human trials [Girard *et al.* 2011]. Compared with other vaccine approaches such as viral vectors and DNA plasmids, protein subunits offer the advantage of considerable experience related to vaccine design and production, since an effective protein subunit vaccine has been developed for influenza A and B infections. Protein subunit vaccines for HIV are based on the HIV envelope. The HIV envelope is composed of glycoproteins, gp120 and gp41, which are cleaved from a gp160 precursor. The mature envelope spike forms as a trimer, composed of three gp120/gp41 complexes. Both recombinant gp160 (rgp160) and recombinant gp120 (rgp120) monomers were studied as immunogens in early HIV vaccine clinical trials. An rgp160 vaccine induced neutralizing antibodies against the homologous vaccine strain but not against heterologous strains, and stimulated limited antibody responses in general [Dolin *et al.* 1991; Keefer *et al.* 1994]. An rgp120 vaccine demonstrated somewhat improved immunogenicity in a phase I trial, including some neutralizing activity against a heterologous strain [Schwartz *et al.* 1993]. A similar rgp120 immunogen derived from a different HIV-1 strain (MN) conferred protection against heterologous strains in chimpanzees [Berman *et al.* 1996], and was found to be safe and immunogenic in humans [Migasena *et al.* 2000]. This immunogen served as the basis for the AIDSVAX vaccines used in the VAX004 and VAX003 trials.

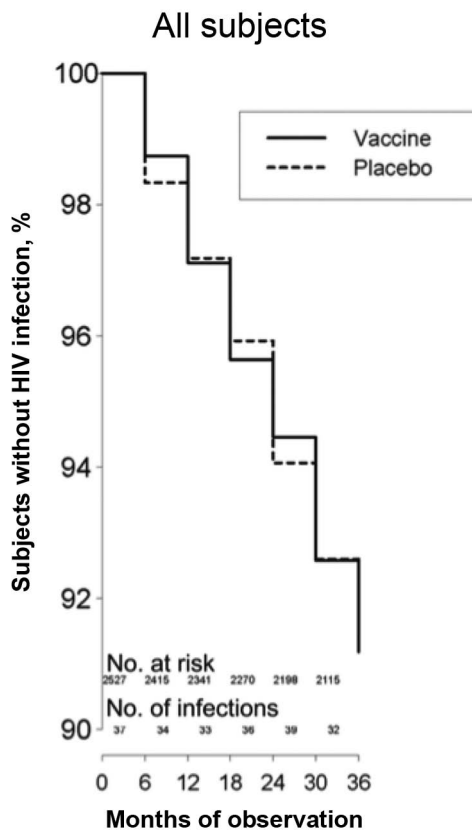


Figure 2. Kaplan–Meier curve from VAX003 showing time to human immunodeficiency virus type 1 (HIV-1) infection, $p = 0.99$. (Reproduced from Pitisuttithum *et al.* [2006] with permission from Oxford University Press.)

VAX004 was the first HIV vaccine efficacy trial and began enrollment in 1998. It was conducted mostly in high-risk men who have sex with men (MSM) in North America and the Netherlands, and evaluated the AIDSVAX B/B vaccine. AIDSVAX B/B contained rgp120 immunogens from strains MN and GNE8. VAX003, which began enrollment in 1999, was conducted in injection drug users in Thailand. It evaluated AIDSVAX B/E, which contained rgp120 immunogens from strains MN and A244. MN was a laboratory-adapted strain, while GNE8 and A244 were primary isolates. The primary isolates were added as a result of evidence demonstrating that laboratory-adapted viruses differ from primary isolates in a number of respects, including the use of the CXCR4 coreceptor rather than CCR5, and increased sensitivity to neutralization. Efficacy against acquisition of infection was not demonstrated in either trial (Figures 1 and 2) [Flynn *et al.* 2005; Pitisuttithum *et al.* 2006]. The

vaccines used in both trials elicited antibodies capable of neutralizing tier 1 viruses, which are sensitive to neutralization, but had little activity against tier 2 viruses, which are more resistant to neutralization [Gilbert *et al.* 2010; Montefiori *et al.* 2012]. These trials demonstrated that rgp120 monomers elicited limited neutralizing antibody responses and failed to protect against HIV infection in a high-risk population.

Current HIV protein subunit immunogens aim to elicit improved neutralizing antibody responses by more accurately representing the native viral envelope. The rgp120 monomers used in the VAX trials had the advantage of being relatively easy to produce, but lacked conformational structures of the native envelope trimer. Many of the critical epitopes that are targets of neutralizing antibodies, including the CD4-binding site, are conformational in nature and highly dependent on the three-dimensional structure of the envelope trimer [Gorny *et al.* 2005; Pantophlet and Burton, 2006; McElrath and Haynes, 2010; Moore *et al.* 2011]. A number of methods have been used in an attempt to stabilize recombinant trimers and thus make them suitable for production on a large scale [Phogat and Wyatt, 2007]. However, most recombinant trimers tested to date have demonstrated only marginally better immunogenicity compared with monomers [Grunder *et al.* 2005; Kim *et al.* 2005; Beddows *et al.* 2007], likely as a result of an inability to accurately represent the characteristics of the native envelope trimer. Another feature of the native viral envelope that may play an important role in the induction of neutralizing antibodies is glycosylation. While HIV envelope glycans have long been thought to play a role in shielding underlying epitopes from neutralizing antibodies, it has been appreciated that these glycans can serve as targets of neutralization as well [Kwong and Mascola, 2012; Moore *et al.* 2012; Lavine *et al.* 2012]. For proper glycosylation of protein subunits, immunogens need to be produced in specific human cell lines [Raska *et al.* 2010]; these cell lines were not utilized to produce the AIDSVAX vaccines. Recently, a stable envelope trimer has been developed that more closely represents antigenic properties of the native envelope trimer, including glycosylation. This trimer has been shown to elicit improved neutralizing antibody responses compared with monomers in guinea pigs [Kovacs *et al.* 2012]. The manufacturing of clinical grade material is under way, and the trimer will likely enter phase I trials in the next 1–2 years.

A recent major advance with important implications for vaccine design has been the detection of highly potent broadly neutralizing antibodies (bnAbs) against HIV-1. Examples include PG9 and PG16, with a breadth of around 80% [Walker *et al.* 2009], VRC-01, with a breadth of around 90% [Wu *et al.* 2010], and 10E8, which neutralizes 98% of tested viruses [Huang *et al.* 2012]. Other bnAbs have been described that do not attain the same degree of neutralization breadth but are almost 10-fold more potent than PG9, PG16, and VRC01 [Walker *et al.* 2011]. Structural modification techniques have also been employed to further enhance the potency and breadth of another bnAb, VRC07 [Kwon *et al.* 2012]. The bnAbs described to date share a number of characteristics. All recognize one of four different sites on the viral envelope spike: the CD4 binding site on gp120, the first and second variable regions (V1/V2) on gp120, the glycan-V3 site on gp120, or the membrane-proximal external region of gp41. In addition, all bnAbs have unusual features: either an uncommonly long complementarity-determining region, extensive somatic mutation, or both [Burton *et al.* 2012; Kwong and Mascola, 2012]. Passive immunization with bnAbs has been shown to confer robust protection against chimeric simian/human immunodeficiency virus (SHIV) infection in NHPs [Moldt *et al.* 2012; Hessel *et al.* 2009, 2010].

Novel strategies will likely be required to develop vaccines capable of stimulating bnAbs against HIV-1. The unusual features of bnAbs described above are highly atypical and only rarely occur in the course of ordinary antibody production. In addition, a number of bnAbs are polyreactive to host antigens, which serves as yet another obstacle to their development [McElrath and Haynes, 2010]. Even in the setting of chronic infection with HIV-1, bnAbs only arise in 10–30% of individuals after a period of 2–4 years [Gray *et al.* 2011a; Mikell *et al.* 2011; Moore *et al.* 2011]. The ability of traditional vaccine strategies to stimulate bnAbs is therefore uncertain, since the immune system may not generate such antibodies without extensive somatic mutation or may suppress the production of these antibodies. A new strategy termed B-cell-lineage vaccine design seeks to increase the elicitation of bnAbs by driving antibody responses along the desired B-cell maturation pathway [Haynes *et al.* 2012b]. B-cell-lineage vaccine design consists of identifying B cells that produce bnAbs and then inferring how those cells evolved from their naïve B-cell ancestor. Vaccine immunogens would then

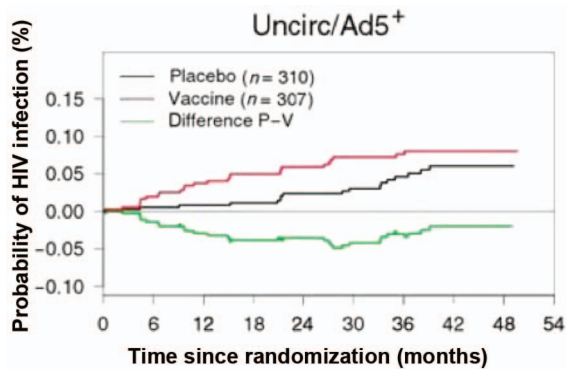


Figure 3. Kaplan–Meier curve from extended follow-up of the Step study, showing time to human immunodeficiency virus type 1 (HIV-1) infection in adenovirus serotype 5 (Ad5)-seropositive, uncircumcised (uncirc) men. $p = 0.02$ for the first 18 months of follow up. [Reproduced from Duerr *et al.* [2012] with permission from Oxford University Press.]

be designed to direct B-cell maturation accordingly. Interestingly, the antigen that stimulates the mature B cell may differ from the antigen that initially activated the naïve B cell. Similarly, different antigens may be required at each stage of B-cell development, resulting in a vaccination strategy consisting of a series of different immunogens. The evolution of the bnAb CH103 in a chronically infected individual has recently been detailed and may serve as a first step to guide the development of a vaccine based on the B-cell-lineage approach [Liao *et al.* 2013]. Another possible approach to increase the probability of eliciting bnAbs involves the use of adjuvants to activate enzymes that regulate somatic mutation. Activation-induced cytidine deaminase (AID) in particular has been extensively studied and may serve as a target for such an approach [Alt *et al.* 2013].

Viral vector vaccines: step and the cellular immune response

Cellular immunity has been shown to play an important role in the immune response against HIV infection. This was demonstrated in simian immunodeficiency virus (SIV) infection in rhesus macaques, where depletion of CD8⁺ T cells by administration of an anti-CD8 monoclonal antibody to chronically infected macaques resulted in a marked increase in viremia and disease progression [Schmitz *et al.* 1999]. Studies of elite controllers of HIV-1 infection have also shown the importance of cellular immunity in controlling viremia. Certain human leukocyte antigen (HLA)

alleles, particularly HLA-B*57, HLA-B*27, and HLA-B*5701, have been correlated with the ability to control viral replication [Carrington and O'Brien, 2003], consistent with the importance of T cells in this effect. Indeed, differences in HLA-B*27-restricted CD8⁺ T cells between individuals who control disease and those who do not have recently been reported [Chen *et al.* 2012]. Of note, natural killer cells may also play a role in the HLA-mediated control of viral replication [Fadda *et al.* 2011], and it may be important to investigate the natural killer cell response as well in the assessment of immunogenicity of vaccine candidates. An approach to elicit a cellular immune response by vaccination is the use of recombinant viral vectors, in which a virus is engineered to express a gene of interest. Viral vectors tested as HIV vaccine candidates include viruses that are replication incompetent or poorly competent of replication in mammalian cells (canarypox and fowlpox), or viruses that have been made replication incompetent or poorly competent through the deletion of genes or through *in vitro* adaptation [adenoviruses, New York Vaccinia virus strain (NYVAC), and Modified Vaccinia Ankara (MVA)]. Of these vectors, recombinant adenovirus serotype 5 (rAd5) was found to be particularly immunogenic, and was selected as the vector for the Step and HVTN 503/Phambili trials, which were the first efficacy trials to evaluate an HIV vaccine designed to stimulate T-cell responses.

Both Step and HVTN 503/Phambili evaluated the same vaccine, an rAd5 vector expressing genes for Gag, Pol, and Nef from clade B viruses. Step was conducted primarily in high-risk MSM in North and South America, the Caribbean, and Australia. The trial was stopped at the first interim analysis after meeting prespecified futility boundaries for efficacy. In addition, a trend towards increased rates of HIV infection was found in male vaccinees who were Ad5 seropositive at baseline, uncircumcised, or both [Buchbinder *et al.* 2008]. In extended follow-up, men who were both uncircumcised and Ad5 seropositive were found to have a statistically significant increased risk of HIV infection, which waned with time from vaccination (Figure 3) [Duerr *et al.* 2012]. The mechanism behind the increased rates of infection in these subjects remains unclear [Barouch, 2010; Duerr *et al.* 2012]. While the rAd5 vaccine used in Step was found to induce CD8⁺ T-cell responses in a majority of vaccinees, the responses were weak and only directed against a limited number of epitopes [McElrath *et al.* 2008]. In vaccinees who

became infected, no effect on viral load, CD4 count, or AIDS-free survival was demonstrated after 4 years. However, in a subset of infected subjects with protective HLA alleles, mean viral load was lower over time in vaccine recipients compared with placebo recipients [Fitzgerald *et al.* 2011]. The mechanism behind this effect is uncertain, but it raises the possibility that individuals expressing protective alleles may benefit from the vaccine. A second trial, HVTN 503/Phambili, was conducted in primarily low-risk heterosexuals in South Africa. Enrollment was halted after increased rates of HIV infection were observed in Step, and only 801 of a planned 3000 participants were enrolled [Gray *et al.* 2011b]. A higher rate of HIV infections was observed in vaccinees compared with placebo recipients, but the difference was not statistically significant.

Following the results of Step, vectors based on other adenovirus serotypes with lower seroprevalence rates than Ad5 have been evaluated. Recombinant vectors using adenovirus serotype 26 (Ad26) and adenovirus serotype 35 (Ad35) were found to be immunogenic in phase I studies [Baden *et al.* 2012; Keefer *et al.* 2012]. In addition to adenovirus vectors, a number of poxvirus vectors have also been evaluated. A canarypox vector was found to have limited immunogenicity in phase I and II trials [Goepfert *et al.* 2005; Russell *et al.* 2007]. NYVAC and MVA appear to be more immunogenic than canarypox and are currently being evaluated in multiple early-phase trials [Gómez *et al.* 2012]. Studies in animal models using combinations of MVA, rAd26, and rAd35 have generally demonstrated improved protection against infection and superior cellular immune responses compared with homologous regimens [Barouch *et al.* 2012; Ratto-Kim *et al.* 2012]. Although only HIV vaccines based on viral vectors that are incapable or poorly capable of replication have been studied in clinical trials to date, replication-competent vectors may be more immunogenic and are also being investigated [Excler *et al.* 2010]. Another avenue of research into vaccines designed to elicit a CD8⁺ T-cell response has focused on the difference between effector memory and central memory CD8⁺ T cells. HIV vaccine candidates have generally elicited a central memory T-cell response, but it has been hypothesized that an effector memory T-cell response may be able to better suppress viremia following infection. Effector memory T-cell responses are primarily elicited through the presence of persistent infections, such as infection by cytomegalovirus

(CMV). In a recent study, a CMV-vectored vaccine was administered to rhesus macaques that were then challenged with a highly pathogenic SIV strain, SIVmac239. Following repeated mucosal challenges, the majority of macaques that received the CMV-vectored vaccine demonstrated profound control of viremia, compared with none of the macaques that received a DNA/rAd5 vaccine [Hansen *et al.* 2012].

Heterologous prime-boost regimens: RV144 and a description of non-neutralizing antibodies

Heterologous prime-boost regimens seek to augment and broaden immune responses by combining different vaccine strategies. This approach was employed in RV144, in which a canarypox viral vector prime expressing Env, Gag, and Pol (ALVAC) was followed by an AIDSVAX B/E boost (the same protein subunit vaccine used in VAX003). The vaccines were administered in Thailand primarily to low-risk heterosexual men and women. Vaccine efficacy against acquisition of infection was found to be 31.2% in the modified intention-to-treat analysis (Figure 4) [Rerks-Ngarm *et al.* 2009]. In vaccinees who did become infected, no effect on CD4⁺ T-cell counts or viral load was observed [Rerks-Ngarm *et al.* 2012]. A subsequent analysis suggested that vaccine efficacy was significantly correlated with risk: efficacy was 68% for participants who maintained low or medium risk throughout the study, but only 5% in the high-risk group [Robb *et al.* 2012]. In addition, efficacy appeared to be highest during the first 12 months and then fell rapidly. In an analysis of immune correlates of risk, binding of immunoglobulin G (IgG) to V1/V2 was associated with a reduced risk of infection, while plasma IgA directed against Env abrogated this protection [Haynes *et al.* 2012a]. This latter effect may have been a result of the interference of IgA with IgG effector functions, which has previously been observed in the setting of both infection and cancer [Griffiss and Goroff, 1983; Jarvis and Griffiss, 1991; Mathew *et al.* 1981]. Indeed, it has recently been reported that some Env-directed IgA antibodies isolated from RV144 vaccinees are capable of blocking IgG-mediated antibody-dependent cellular cytotoxicity (ADCC) effector function [Tomaras *et al.* 2013]. In additional immunogenicity analyses, the RV144 regimen was found to elicit a weak neutralizing antibody response [Montefiori *et al.* 2012], and only a moderate T-cell response, although the CD4⁺ T-cell

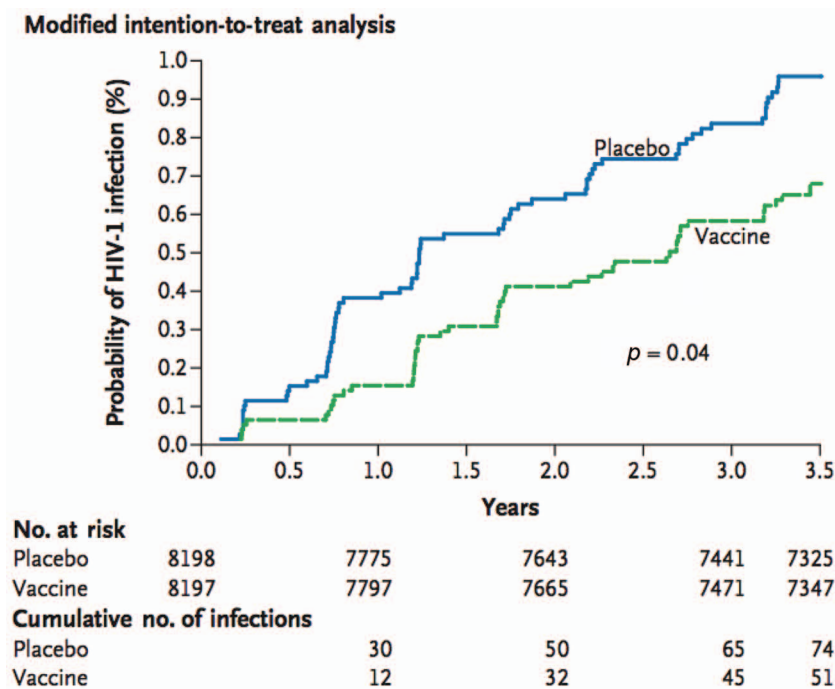


Figure 4. Kaplan–Meier curve from RV144 showing time to human immunodeficiency virus type 1 (HIV-1) infection in the modified intention-to-treat analysis. (Reproduced from Rerks-Ngarm *et al.* [2009] with permission of the Massachusetts Medical Society.)

response that was elicited was directed against V2 [de Souza *et al.* 2012]. The possible effect of the immune response against V2 was also demonstrated by an analysis of breakthrough viral strains: vaccine efficacy against viruses matching the vaccine at amino acid position 169 was 48%, and vaccine efficacy against viruses mismatching the vaccine at position 181 was 78% [Rolland *et al.* 2012]. Both of these amino acids are located within the V2 region of Env.

The immune correlates analyses of RV144 did not identify neutralizing antibody or cellular immune responses that were correlated with reduction of risk, which raises the possibility that non-neutralizing antibodies against V1/V2 may play a role in protection. Following the results of RV144, non-neutralizing antibodies against V2 were also found to correlate with protection against a stringent SIV challenge in rhesus macaques vaccinated with DNA/MVA, rAd26/MVA, or MVA/rAd26 regimens [Barouch *et al.* 2012]. While the mechanism of protection in these trials remains unclear, non-neutralizing antibodies against V1/V2 may confer protection through effector functions such as ADCC, which occur in conjunction with cells of the innate immune system [Robinson, 2013]. The four IgG

subclasses differ in their ability to mediate these effector functions, and IgG3 generally has the greatest activity [van de Winkel and Anderson, 1991]. Interestingly, while the antibody response in RV144 was weaker overall than VAX003, preliminary data suggest that the response in RV144 was skewed towards IgG3, which was not the case in VAX003 [Chung *et al.* 2012].

DNA vaccines and HVTN 505

DNA vaccines consist of a plasmid encoding a protein of interest. A DNA vaccine can deliver the same genes as a live-vectored vaccine without immunity developing against the vector, which may inhibit expression of the insert [Donnelly *et al.* 1997]. While DNA vaccines in general demonstrated promise in animal models, early candidate DNA vaccines were found to be poorly immunogenic when tested in humans for a number of viruses, including HIV [MacGregor *et al.* 1998; Ferraro *et al.* 2011]. Later generation DNA vaccines have incorporated various strategies to improve immunogenicity, including electroporation and the use of molecular adjuvants [Baden *et al.* 2011]. A number of DNA HIV vaccine candidates are currently being evaluated, mostly in combination with viral vectors in heterologous

prime-boost regimens. In a phase I trial, a heterologous DNA/rAd5 regimen demonstrated improved CD4⁺ T-cell responses and increased CD8⁺ T-cell interleukin 2 production compared with a homologous rAd5/rAd5 regimen [Cox *et al.* 2008]. In another phase I trial, a DNA/rAd5 regimen elicited significantly improved T-cell and antibody responses compared with either DNA or rAd5 alone [Koup *et al.* 2010]. A DNA/MVA regimen was similarly found to improve the T-cell immune response compared with MVA alone, although interestingly the antibody response was superior when MVA was used without DNA priming [Goepfert *et al.* 2011].

The most recently conducted HIV vaccine efficacy trial, HVTN 505, is a phase IIb trial that evaluated a DNA prime followed by a rAd5-vec-tored boost in MSM in the United States. The DNA plasmid expressed Gag, Pol, Nef, and Env, and the rAd5 boost expressed Gag, Pol, and Env. This was in contrast to the rAd5 vaccine used in Step, which did not express Env. In an attempt to avoid the possibility of increased risk of HIV infection that occurred using the rAd5 vector in Step, only men who were circumcised and Ad5 seronegative were eligible for inclusion. The HVTN 505 trial enrolled 2504 subjects, but on 25 April 2013, the HVTN announced that the trial was halted for lack of efficacy because futility criteria were met for both primary endpoints: HIV acquisition and postacquisition viral load setpoint (the press release can be viewed at <http://hvtn.org/505-announcement-25April2013.html>). At the time the trial was halted, 27 HIV infections had occurred in vaccine recipients compared with 21 HIV infections in placebo recipients; however, the rates of HIV infections in vaccinees and placebo recipients were not statistically significantly different. Participants in the HVTN 505 trial will be followed closely for additional study endpoints. Further data and analyses should be forthcoming and may aid in the interpretation of the primary results.

Insights from the transmission event

The transmission of HIV-1 and the properties of transmitted viruses are being studied in detail to determine whether such information can inform development of an HIV vaccine. During chronic infection, a quasispecies of virus resides within a single individual [Korber *et al.* 2001]. However, studies of sexual transmission have found that in approximately 80% of cases only a single viral

strain is transmitted [Keele *et al.* 2008; Abrahams *et al.* 2009]. Investigation of this 'bottleneck' may identify viral properties that affect the ability of a virus to establish infection. Indeed, a number of studies have found that transmitted viruses, also known as founder viruses, appear to have particular features. In an early study of three mother–infant pairs, the infants' viral sequences were less diverse than the mothers', and a conserved N-linked glycosylation site within the V3 region found in each of the mothers' sequences was absent in the infants' sequences [Wolinsky *et al.* 1992]. Transmitted clade C viruses were found to have shorter, less glycosylated envelope variable loops than viruses present during chronic infection, and were more sensitive to neutralization [Derdeyn *et al.* 2004]. In addition, the glycan at amino acid position 332 may be significantly underrepresented in transmitted clade C viruses compared with viruses present during chronic infection [Moore *et al.* 2012]. In a cohort of clade D and A transmission pairs, transmitted strains appeared to be more closely related to variants found early during the donor's course of infection rather than to variants circulating in the donor near the time of transmission [Redd *et al.* 2012].

The above findings are complicated by the fact that both the mode of transmission and viral clade appear to play a role in the determination of the number of transmitted viruses and their particular features. While only a single variant is transmitted in approximately 80% of heterosexual transmission events, only 46% of transmitted viruses appeared to derive from a single variant in a cohort of MSM [Gottlieb *et al.* 2008]. A similar finding was also noted for MSM who were included in the study by Keele and colleagues cited above. In rhesus macaques, intravenous inoculation of SIV resulted in a more complex founder population than intravaginal inoculation [Greenier *et al.* 2001]. Even the bottleneck present during heterosexual transmission may not operate under certain conditions. A study conducted in a small heterosexual African cohort found that a single viral strain was transmitted in the vast majority of cases; however, more viral strains appeared to be transmitted in the presence of genital infection [Haaland *et al.* 2009]. This suggests that an intact mucosal barrier may be necessary to restrict the genetic diversity of the infecting virus. While the particular characteristics found in clade C transmitted viruses were also found in clade A viruses, the same characteristics were not noted in clade B or D viruses

[Chohan *et al.* 2005; Frost *et al.* 2005; Sagar *et al.* 2009]. A specific viral signature that distinguishes transmitted viruses across all clades and modes of transmission has yet to be found. If transmitted viruses are in fact appreciably different from other HIV strains, future vaccines could be designed to particularly target these viruses.

The mosaic sequence insert

The diversity of HIV presents a significant challenge to the ultimate goal of creating a global vaccine. In an attempt to address this problem, mosaic sequences designed for insertion into viral vector vaccines have been developed. These genetic sequences were created using computer algorithms to maximize the coverage of potential T-cell epitopes from worldwide HIV-1 strains [Fischer *et al.* 2007]. T-cell epitopes are amino acid sequences presented on the surface of infected cells by HLA class I molecules and recognized by CD8⁺ T cells. By maximizing the representation of global viral strains, mosaic sequences may elicit immune responses capable of recognizing viruses from multiple clades. Studies in rhesus macaques have shown that mosaic sequences increase the breadth and depth of the T-cell response compared with consensus or natural sequences [Barouch *et al.* 2010; Santra *et al.* 2010]. However, mosaic sequences have not yet been evaluated in humans, and the immune response they will elicit remains unknown. Phase I clinical trials using mosaic sequence inserts in orthopox-vectored and adenovirus-vectored vaccines are planned in the coming year.

Vectored immunoprophylaxis

Vectored immunoprophylaxis is an approach that provides bnAbs by employing a viral vector containing inserts of immunoglobulin genes. This bypasses the difficult task of utilizing immunogens to elicit these unusual antibodies. The vector employed in a study of this approach was adeno-associated virus (AAV), into which genes were inserted that code for a bnAb against HIV-1. AAV has been extensively studied as a potential gene therapy vector because it is not known to cause disease and has been engineered so that it does not integrate into the human genome. Intramuscular administration of an AAV vector with human immunoglobulin genes into a humanized mouse resulted in prolonged expression of bnAbs and conferred protection against high-dose intravenous HIV challenges [Balazs *et al.* 2012]. The AAV

vector is relatively inexpensive to produce and offers the possibility of long-lasting protection. However, this approach also presents concern about the ability to halt gene expression if toxicities related to immunoglobulin production were to occur. Methods to address this concern are currently being investigated.

Conclusion

Only four HIV vaccine concepts have undergone efficacy trials, and while only one has demonstrated efficacy, all four have generated important information. The VAX004/VAX003 trials indicated that a subunit vaccine composed of recombinant gp120 monomers was not effective in a high-risk population. An Ad5 vector vaccine failed to confer protection in the Step trial and appeared to be associated with an increased risk of HIV-1 infection in certain populations. The addition of a DNA prime and the inclusion of genes expressing Env to the Ad5 vector in HVTN 505 also failed to confer protection. A prime-boost regimen consisting of a canarypox vector prime and an rgp120 boost (RV144) demonstrated modest efficacy (31.2%), albeit for a short period of time. While the components of the immune response responsible for protection in RV144 are yet to be fully determined, initial studies have suggested that an antibody response to the envelope V1/V2 region may have played a role. Genetic sequences in the V2 region were also found to be associated with vaccine efficacy in an analysis of breakthrough viral strains. Interestingly, vaccination in both VAX003 and Step appeared to impact the sequences of breakthrough HIV strains as well [Shmelkov *et al.* 2011; Rolland *et al.* 2011]. This suggests that the vaccines used in those trials may have placed some selective pressure on transmitted viruses. The correlation between an HIV vaccine's ability to exert genetic pressure on transmitted viral strains and its ability to confer protection remains to be determined.

RV144 demonstrated efficacy in a low-risk heterosexual population; however, no other trial to date has been completed in such a population (Table 1). The role of population risk factors in the ultimate success of the regimen used in RV144 is therefore unclear. As discussed above, a single viral strain is transmitted in the majority of heterosexual transmission events. Thus, a candidate vaccine administered to this population may only be required to inhibit a single viral strain, which could potentially enhance its effectiveness. The

role of neutralizing antibodies in the protective effect observed in RV144 also remains unclear. Although the generation of neutralizing antibodies appeared to be weak, it is possible that a low level of neutralizing antibodies was able to confer protection in this particular setting [Bar *et al.* 2012].

Further studies are planned to confirm and extend the results of RV144. These studies will also evaluate viral-vector prime and protein-subunit boost regimens, and will incorporate novel vectors, inserts, and protein subunits. Vectors such as Ad26, MVA, and NYVAC have been found to be highly immunogenic, and future trials will evaluate these vectors both alone and in combination. The mosaic sequence insert, which may elicit a broader immune response than natural HIV-1 sequence inserts, will also be studied in upcoming trials. A novel recombinant glycoprotein trimer will be evaluated as a protein boost, and may elicit a more potent neutralizing antibody response than rgp120 monomers. Because the protection observed in RV144 was of limited duration, future studies evaluating similar regimens may incorporate extended immunization schedules, particularly to stimulate antibody responses, in an attempt to increase the duration of protection.

The discovery of highly potent bnAbs against HIV-1 is an observation of great importance for the development of an HIV vaccine. However, traditional vaccine strategies may be unable to elicit these antibodies. Novel strategies such as B-cell-lineage vaccine design and vectored immunoprophylaxis are being studied to elicit or provide these antibodies.

The HIV vaccine efficacy trials conducted to date have demonstrated that a safe and effective HIV vaccine is possible, and have made important contributions to our understanding of the path towards the development of such a vaccine. Future progress will depend on an iterative relationship between findings from preclinical studies and from properly designed, efficiently conducted clinical trials.

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Dr Dolin is a consultant for Visterra, a biotechnology company.

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