Published in final edited form as:
Nature. 2008 October 2; 455(7213): 613–619. doi:10.1038/nature07352.

Challenges in the Development of an HIV-1 Vaccine

Dan H. Barouch
Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

Abstract

The development of a safe and effective HIV-1 vaccine is a critically important global health priority. Despite recent advances in our understanding of HIV-1 pathogenesis and immunology, however, major scientific obstacles remain. Prototype HIV-1 vaccine candidates aimed at eliciting humoral and cellular immune responses have to date failed to protect against HIV-1 infection or to reduce viral loads following infection in clinical efficacy studies. A renewed and coordinated commitment to basic discovery research, preclinical studies, and clinical trials will therefore be required to overcome the hurdles currently facing the field. Here I review key challenges and future prospects in the quest to develop a prophylactic HIV-1 vaccine.

It has been 25 years since HIV-1 was identified as the causative agent for AIDS[1–5]. Over 60 million people worldwide have been infected with HIV-1, mostly in the developing world, and nearly half of these individuals have died. The development of a safe and effective HIV-1 vaccine would undoubtedly be the best solution for the ultimate control of the worldwide AIDS pandemic[6], but unfortunately HIV-1 vaccine development efforts have not yet proven successful. The extraordinary diversity of HIV-1, the capacity of the virus to evade adaptive immune responses, the inability to induce broadly reactive antibody responses, the early establishment of latent viral reservoirs, and the lack of clear immune correlates of protection represent unprecedented challenges for vaccine development.

The goal of an HIV-1 vaccine would be either to prevent infection or to reduce viral loads and clinical disease progression following infection (Fig. 1). An ideal vaccine would completely block infection and provide sterilizing immunity. Although such a vaccine would be optimal, this degree of protection is not even achieved with the majority of clinically licensed vaccines. In contrast, most licensed viral vaccines appear to function by controlling subclinical viral replication and by preventing clinical disease. It may therefore be more realistic to develop a suboptimal HIV-1 vaccine that fails to prevent infection but that provides partial immune control of viral replication following infection. Such partial control, as exemplified by a reduction in peak and setpoint viral loads following infection, has been demonstrated in certain preclinical studies by vaccines that elicit T lymphocyte responses. Moreover, since viral loads represent a principal determinant of HIV-1 transmission[7], it is conceivable that such a partially protective vaccine might have substantial impact on a population level.

Despite the urgent need for an HIV-1 vaccine, only two vaccine concepts have completed clinical efficacy studies to date. The first vaccine concept utilized monomeric HIV-1 Env gp120 protein vaccine candidates, and the aim of this strategy was to induce Env-specific humoral immune responses. In early phase clinical trials, gp120 immunogens elicited type-specific binding antibodies but failed to induce broadly reactive neutralizing antibodies (NAbs)[8, 9]. In

Contact information: E/CLS – 1047, Division of Viral Pathogenesis, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Boston, MA 02215, USA; Tel: 617-735-4485; Fax: 617-735-4527; Email: dbarouch@bidmc.harvard.edu.

The author declares no competing financial interests.

two phase 3 efficacy trials sponsored by the biotechnology company VaxGen, these vaccine candidates afforded no detectable protective efficacy[10, 11], indicating that these type-specific antibody responses were insufficient to protect against HIV-1 infection in humans. Another phase 3 study evaluating the efficacy of a recombinant canarypox vector prime, gp120 protein boost vaccine regimen is currently underway. The second vaccine concept that has completed clinical efficacy studies involved replication-incompetent, recombinant adenovirus serotype 5 (rAd5) vectors expressing HIV-1 Gag, Pol, and Nef. The aim of this strategy was to elicit HIV-1-specific cellular immune responses. Early phase clinical trials demonstrated that rAd5 vector-based vaccines elicited cellular immune responses in the majority of subjects, although these responses were partially suppressed in individuals with pre-existing Ad5-specific NAbs[12]. Phase 2b efficacy trials sponsored by Merck and the National Institutes of Health were unexpectedly terminated when the first planned interim analysis showed that this vaccine failed to protect against infection or to reduce viral loads following infection and that vaccinees with pre-existing Ad5-specific NAbs exhibited an enhanced rate of HIV-1 acquisition[13]. These results have highlighted new scientific challenges and have led to substantial debate regarding the optimal path forward for the HIV-1 vaccine field.

Virologic and Immunologic Challenges

The challenges in the development of a prophylactic HIV-1 vaccine are unprecedented (Box 1). The extraordinary worldwide diversity of HIV-1 presents perhaps the greatest hurdle[14]. Driven by the error-prone reverse transcriptase, the HIV-1 M group has diversified into nine divergent clades as well as multiple circulating recombinant forms. Amino acid sequences of Env can differ up to 20% within a particular clade and over 35% between clades[14, 15]. A vaccine immunogen will therefore need to contend with a remarkably high degree of viral diversity, and vaccine protection will necessarily be dependent on the capacity of immune responses to cross-react with highly heterologous viruses. Although cross-reactive humoral and cellular immune responses against conserved regions of the virus have been reported, it is reasonable to assume that protective efficacy will diminish substantially with increasing divergence between vaccine antigens and infecting viruses.

Another key challenge is the lack of clear immune correlates of protection in humans, since HIV-1-infected patients are unable to eradicate the virus. Suggestive evidence regarding immune correlates of protection might be obtained from viral challenge studies in nonhuman primates and from studies of HIV-1-infected individuals who spontaneously control viral replication to very low levels. However, definitive immune correlates of protection will likely only emerge in the context of successful vaccine efficacy studies in humans.

HIV-1-specific humoral immunity

Virus-specific NAb titers represent a key immune correlate for most licensed viral vaccines, and thus early studies focused on developing HIV-1 Env subunit immunogens. Advances in our understanding of Env structure and function have begun to elucidate why generating broadly reactive NAbs to HIV-1 by vaccination may be so difficult[16]. The HIV-1 Env glycoprotein is a trimer on the virion surface with extensive N-linked glycosylation that effectively shields many conserved epitopes from antibody recognition[17, 18]. Highly immunogenic variable loops also elicit type-specific antibodies that may redirect humoral responses away from conserved regions. In addition, key conserved regions, such as the chemokine coreceptor binding site, are only formed after Env binds its cellular receptor CD4 and undergoes an extensive conformational change[19]. The development of mutations in N-linked glycans has also been shown to lead to rapid evasion of host NAb responses[20, 21].

Nevertheless, broadly reactive NAb activity has been identified in a small number of HIV-1-infected subjects, and this reactivity appears largely directed against conserved regions of the

Env glycoprotein such as the CD4 binding site[22]. The broadly reactive mAb b12 also binds to the CD4 binding site, suggesting that this region of Env may represent a critical point of vulnerability that is potentially amenable to neutralization[23]. However, the CD4 binding site is recessed and only partially accessible to antibody binding. Another conserved region is the membrane proximal external region (MPER) of gp41, which represents the target of the broadly reactive mAbs 2F5 and 4E10. However, MPER-specific NAbs may be difficult to elicit by vaccination for multiple reasons, including tolerance control and immunoregulation[24], sequestration of the epitope in the lipid membrane[25], exposure of the epitope only transiently during viral entry[26], or possibly a combination of multiple factors.

The development of immunogens that induce broadly reactive NAbs is perhaps the most important priority for the HIV-1 vaccine field[16]. Proof-of-concept passive transfer studies in nonhuman primates have shown that administration of high doses of broadly reactive mAbs can afford sterilizing protection from infection, thus demonstrating the potential of virus-specific humoral immunity[27, 28]. However, it has not been possible to induce such broadly reactive NAbs by vaccination to date. Although there has been substantial progress in our understanding of Env structure and function, there are currently no vaccine candidates that are aimed at eliciting broadly reactive Env-specific NAbs in clinical trials. It is likely that next generation Env immunogens will need to be engineered antigens. Strategies that are being pursued include generating biochemically stabilized Env trimers, constraining Env immunogens in structurally defined conformations, scaffolding conserved neutralization epitopes onto foreign proteins, developing methods to circumvent immunoregulation, and designing immunogens to target specific regions such as the CD4 binding site, the MPER region, and structurally conserved elements of the V3 loop. The relevance of non-neutralizing antibodies that mediate other effector functions such as antibody-dependent cell-mediated virus inhibition, complement activation, and phagocytosis is also being investigated.

HIV-1-specific cellular immunity

Virus-specific T lymphocyte responses are believed to play a critical role in controlling HIV-1 replication and are therefore being actively explored in vaccine development strategies. Early studies showed that virus-specific CD8+ T lymphocyte responses emerge during acute infection coincident with initial control of primary viremia[29–31]. Potent cellular immune responses have also been reported in long-term nonprogressors[32], and specific HLA alleles and the breadth of Gag-specific T lymphocyte responses have been correlated with control of viral replication in HIV-1-infected individuals[33, 34]. These data indicate the potential importance of cellular immune responses in immune control of HIV-1. Concordant with these observations, experimental depletion of CD8+ T lymphocytes has been shown to abrogate immune control of simian immunodeficiency virus (SIV) replication in rhesus monkeys[35, 36].

A limitation of virus-specific T lymphocyte responses is the propensity of the virus to accumulate mutations in T lymphocyte epitopes and to evade cellular immune control[37–39]. It is therefore likely that the breadth of epitope-specific T lymphocyte responses will prove critical for an HIV-1 vaccine, not only to maximize immunologic coverage of HIV-1 diversity but also to minimize the potential for viral escape from recognition by T lymphocytes. However, the breadth of vaccine-elicited cellular immune responses may be limited by immunodominance constraints and the inherent tendency of CD8+ T lymphocyte responses to be highly focused on a limited number of epitopes.

Recent advances in the characterization of T lymphocyte responses by multiparameter flow cytometry have highlighted the functional diversity of virus-specific T lymphocytes in terms of cytokine secretion, degranulation, proliferation, and other effector functions in various subpopulations of effector and memory T lymphocytes. It is likely that the complex

functionality of T lymphocytes may ultimately prove more relevant than IFN-γ secretion as measured by ELISPOT assays for the evaluation of vaccine-elicited cellular immune responses. Polyfunctional T lymphocytes capable of performing multiple functions have been reported in long-term nonprogressors[40], in recipients of effective vaccines such as vaccinia[41], and in certain preclinical challenge studies[42]. These considerations suggest that the breadth[43] and quality[44] of T lymphocyte responses may prove critical in addition to the magnitude of these responses.

Perhaps the most significant limitation of vaccine-elicited cellular immune responses is that they will not likely protect against acquisition of infection. As a result, vaccine-induced T lymphocyte responses will presumably be unable to prevent lifelong infection, since the virus rapidly establishes latent reservoirs[45, 46]. Moreover, it is unclear whether vaccine-elicited T lymphocytes will be able to function rapidly enough given that important immunopathologic events occur within the first few days of acute HIV-1 infection. HIV-1 preferentially infects HIV-1-specific CD4+ T lymphocytes[47] and rapidly depletes the majority of memory CD4+ T lymphocytes in gut-associated lymphoid tissue (GALT) within the first 4–10 days of infection[48–50]. This sets the stage for progressive immunodeficiency as well as for chronic immune activation, which likely results at least in part from microbial translocation across damaged gastrointestinal mucosa[51]. Given the time required for vaccine-induced CD8+ T lymphocyte responses to expand following infection, it may be difficult for vaccine-elicited T lymphocytes to prevent these early immunopathologic events completely[52].

Current HIV-1 Vaccine Strategies

Traditional strategies

Vaccine strategies for HIV-1 can be divided into traditional and novel vaccine approaches (Box 2). Traditional vaccine technologies include live attenuated viruses, whole killed viruses, and protein subunits. Although these approaches have proven enormously successful for the development of vaccines against other viruses, they all have substantial limitations in terms of their utility for HIV-1. Live attenuated viruses have afforded substantial protective efficacy against SIV challenges in rhesus monkeys[53, 54] but are unlikely to be utilized in humans due to significant safety concerns[55–57]. In contrast, whole killed viruses[58] and protein subunits[10, 11] are limited by their inability to induce broadly reactive NAb responses as well as by their inability to elicit CD8+ T lymphocyte responses. Recent data, however, suggests that Toll-like receptor (TLR) adjuvants may increase the utility of protein subunit immunogens[59, 60].

Novel strategies

Novel vaccine strategies include gene delivery technologies such as plasmid DNA vaccines and live recombinant vectors that are engineered to express HIV-1 antigens. Plasmid DNA vaccines offer considerable promise in terms of simplicity and versatility, but multiple injections of high doses of DNA vaccines are typically required to elicit detectable immune responses in nonhuman primates and humans[61, 62]. Substantial research is therefore focused on the development of adjuvants for DNA vaccines[63, 64] and improved delivery technologies such as in vivo electroporation[65, 66]. Recombinant vectors include attenuated or replication-incompetent viruses, most notably adenoviruses[12, 67, 68] and poxviruses[69, 70]. Viral vectors, administered either alone or in the context of heterologous DNA prime, vector boost regimens, represent the majority of HIV-1 vaccine candidates that are currently in clinical trials. Other viral vectors that are being evaluated include vesicular stomatitis virus (VSV), adeno-associated virus (AAV), Venezuelan equine encephalitis (VEE) virus, cytomegalovirus (CMV), herpes simplex virus (HSV), and measles virus. Bacterial and mycobacterial vectors are also being explored, including *Salmonella*, *Listeria*, and BCG.

The STEP Study

Preclinical background

Recombinant Ad5 vectors were selected for development by Merck based on preclinical vector comparison studies that showed that rAd5 vectors were more immunogenic than multiple other vector modalities in rhesus monkeys[67, 71]. Moreover, rAd5 vectors expressing SIV Gag afforded dramatic reductions of viral loads following challenge of rhesus monkeys with the chimeric simian-human immunodeficiency virus (SHIV) 89.6P[67]. However, it was also observed that the same vaccine afforded minimal to no control of peak or setpoint viral loads following challenge with SIVmac239[72], indicating that SIV challenges were considerably more stringent than SHIV-89.6P challenges.

A DNA prime, rAd5 boost regimen expressing SIV Gag afforded a brief (90 days) and marginal (0.8 log) reduction of peak viral loads following SIVmac239 challenge[72], but this effect was only observed in rhesus monkeys that were selected to express the MHC class I molecule Mamu-A*01 that is associated with efficient virologic control[73–75]. A DNA prime, rAd5 boost regimen expressing multiple SIV antigens afforded increased protective efficacy in Mamu-A*01-positive rhesus monkeys[76], indicating that expanding the breadth of cellular immune responses improves protection. However, neither rAd5 alone nor DNA prime, rAd5 boost regimens have to date been able to reduce setpoint viral loads following SIV challenge of Mamu-A*01-negative rhesus monkeys[72, 77].

Clinical studies

The Merck HIV-1 vaccine candidate was formulated as a trivalent mixture of rAd5 vectors expressing HIV-1 clade B Gag, Pol, and Nef. Phase 1 clinical trials suggested that this vaccine was generally well tolerated and immunogenic in the majority of volunteers[12]. However, as predicted by preclinical studies[61], responses to this vaccine were partially suppressed in individuals with pre-existing NAbs against the vaccine vector. Since 30–40% of individuals in the United States and Western Europe and 80–90% of people in sub-Saharan Africa have pre-existing Ad5-specific NAbs[78–81], the impact of anti-vector immunity was predicted to be a limitation of rAd5 vectors.

Two phase 2b “proof-of-concept” efficacy studies were initiated by Merck and the National Institutes of Health to determine whether HIV-1-specific cellular immune responses induced by this vaccine regimen would prevent HIV-1 infection or would reduce viral loads following infection. HVTN 502, also known as the “STEP” study, was a 3,000 subject study in the Americas, the Carribean, and Australia. HVTN 503, also called “Phambili” (which means “to move forward” in Xhosa), was designed as a parallel 3,000 subject study in South Africa.

On September 18, 2007, HVTN 502 was unexpectedly terminated at the first planned interim analysis when the Data and Safety Monitoring Board declared futility in the study achieving its primary endpoints[13]. Moreover, in subjects with pre-existing Ad5 NAb titers, a greater number of HIV-1 infections occurred in vaccinees as compared with placebo recipients (Fig. 2). Although the biological basis for this observation remains unclear, these data suggest that vaccination with rAd5 vectors may be associated with an increased risk of HIV-1 acquisition in this subgroup. Post-hoc multivariate analysis further suggested that the greatest increased risk was in men who had pre-existing Ad5 NAbs and who were uncircumcised.

It is currently unclear whether the lack of efficacy in the STEP study simply represents the failure of the Merck rAd5-Gag/Pol/Nef vaccine product or whether this might be the harbinger of the failure of the T cell vaccine concept overall. It is likely that substantial data will emerge from detailed immunologic analyses of vaccinees who subsequently became infected, and it is possible that the rAd5-Gag/Pol/Nef vaccine failed to induce sufficient magnitude, breadth,

or quality of cellular immune responses[82]. At the present time, therefore, it would seem premature to consider the failure of this single study as the failure of T cell based vaccines in general.

The apparent increased risk of HIV-1 acquisition in vaccinees with pre-existing Ad5-specific NAbs was unexpected, and this finding highlights our lack of understanding of the parameters that determine susceptibility to HIV-1 infection. The biologic basis for this observation remains unclear. One hypothesis is that anamnestic Ad5-specific CD4+ T lymphocytes following rAd5 vaccination in individuals with pre-existing Ad5-specific NAbs may have been increased targets for HIV-1 infection. However, early data has suggested that Ad5-specific T lymphocyte responses following rAd5 vaccination are actually lower in individuals with pre-existing Ad5-specific NAbs as compared with those without pre-existing Ad5-specific NAbs (J. McElrath, unpublished data). An alternative hypothesis is that Ad5-specific NAbs may have opsonized rAd5 vectors following immunization and could have led to different tropism or inflammatory responses. It is also possible that pre-existing Ad5-specific NAbs may have been a marker for other confounding variables that have not yet been identified.

A STEP Forward?

Despite the disappointing results of the STEP study, several key lessons have already been learned. First, it is clear that the path forward towards an HIV-1 vaccine will be neither simple nor straightforward. Second, the importance of understanding both systemic and mucosal immune responses to vaccine vectors is paramount. Third, the biological determinants of HIV-1 acquisition and the impact that vector-specific and antigen-specific mucosal immune responses may have on this process will require intensive investigation. Fourth, clinical vaccine studies will need to adapt to the safety concerns raised by the STEP study, such as possibly excluding subjects who have pre-existing NAbs to the vaccine vector that is utilized until this phenomenon is more completely understood. Fifth, future T cell based vaccine candidates should be prioritized for clinical efficacy studies only if they are convincingly superior to the homologous rAd5-Gag/Pol/Nef regimen that has failed. Sixth, nonhuman primate challenge models should be recalibrated based on the STEP study to help guide future vaccine development.

The protection afforded by the homologous rAd5 regimen against SHIV-89.6P indicates that this model lacks sufficient stringency for the evaluation of T cell based vaccine candidates. Although the more stringent SIV challenge model cannot be considered to be validated until there is a successful clinical efficacy study in humans, it seems reasonable to utilize SIVmac239 or SIVmac251 as challenge viruses for evaluating next generation vaccine candidates (Box 3). Preclinical challenge studies need to be adequately powered with sufficient follow-up time, and the vaccine schedule and dose should model the proposed clinical regimen. For optimal stringency, studies should exclude rhesus monkeys that express MHC class I alleles that are specifically associated with efficient virologic control, such as Mamu-A*01, Mamu-B*17, and Mamu-B*08. The use of homologous Env antigens that may inappropriately overestimate protective efficacy should also be avoided. Mucosal challenges may offer certain physiologic advantages over intravenous challenges, and these challenge models should therefore be developed. Finally, increased emphasis should be placed on assessing the capacity of promising vaccine candidates to protect against highly heterologous SIV challenges, since infecting viruses in humans will almost certainly be heterologous to any vaccine sequence. Since very few heterologous SIV challenge studies have been performed to date, a practical approach may be to determine the protective efficacy of promising vaccine candidates against both homologous and heterologous SIV challenges. It is currently debated whether nonhuman primate challenge studies should be utilized as a formal “gatekeeper” for advancing vaccine candidates into clinical efficacy studies, since the capacity of this model to predict the results

of clinical efficacy studies remains unclear. Nevertheless, it would seem reasonable to give a relative priority to develop vaccine candidates that lead to durable control of setpoint viral loads following SIV challenge.

The STEP study has had a major impact on other HIV-1 vaccine programs in the field. HVTN 503 was terminated as it utilized the same rAd5 based vaccine candidate that was utilized in HVTN 502. The NIH Vaccine Research Center has developed a DNA prime, rAd5 boost vaccine regimen expressing clade B Gag-Pol and multiclade Env antigens. This vaccine candidate has been shown to be immunogenic in the majority of individuals in phase 1 studies, particularly for the Env antigens[62, 68, 83]. In preclinical studies, a DNA prime, rAd5 boost vaccine regimen expressing SIV Gag, Pol, Nef, and Env antigens afforded a 1.1 log reduction of peak viral loads for 112 days following a homologous SIVmac251 challenge[77]. No durable control of setpoint viral loads was observed with this vaccine, although delayed progression to AIDS-related mortality was evident[77]. NIAID recently announced that it will not proceed with a large phase 2b efficacy study known as PAVE 100, although a smaller, more focused efficacy study with this vaccine candidate is still under consideration[84]. DNA prime, poxvirus boost regimens are also being evaluated utilizing MVA[69] and NYVAC[70] vectors, and phase 1 clinical trials have demonstrated immunogenicity in the majority of volunteers. Central to all of these programs, however, is the hypothesis that DNA priming prior to vector boosting will improve protective efficacy. This has been observed in some[72] but not all[77] SIV challenge studies, and thus it still remains an open question that requires further investigation and should be considered a high priority.

Novel rAd vectors derived from Ad serotypes that are rare in human populations are also being explored as a strategy to evade pre-existing Ad5-specific NAbs. It is hoped that such vectors may offer immunologic as well as safety advantages as compared with rAd5 vectors by circumventing pre-existing vector-specific NAbs. However, these possibilities have not yet been confirmed in clinical trials. Current strategies include the development of rare serotype rAd26, rAd35, and rAd48 vectors[78, 79, 85]; chimeric rAd5HVR48 vectors in which dominant Ad5-specific NAb epitopes have been exchanged[86]; and non-human rAd vectors[87, 88]. Rare serotype rAd vectors are biologically different from rAd5 vectors in terms of their cellular receptors, tropism, intracellular trafficking pathways, and innate immune profiles. Moreover, rAd26 and rAd48 vectors elicit T lymphocyte responses of a substantially different phenotype as compared with rAd5 vectors[89], and potent heterologous rAd prime-boost regimens can be constructed utilizing serologically distinct rAd vectors. We have recently demonstrated that a heterologous rAd26 prime, rAd5 boost regimen expressing SIV Gag afforded a durable 2.4 log reduction of setpoint viral loads following SIVmac251 challenge of Mamu-A*01-negative rhesus monkeys, whereas a homologous rAd5 regimen provided no protection in this stringent challenge model (D. Barouch, unpublished data). These data suggest that vaccine candidates that elicit improved magnitude, breadth, and quality of T lymphocyte responses may provide superior protective efficacy as compared with homologous rAd5 regimens.

Perspectives and Future Directions

To a great extent, HIV-1 vaccine science is still in its infancy. Major unsolved problems remain, and a renewed commitment to basic discovery research in addition to preclinical studies and clinical trials will be required to move the field forward. Clinical trials that are focused on answering specific scientific hypotheses rather than exclusively aimed at product development may be most useful to the field at the present time. Certain vaccine regimens, such as heterologous rAd prime-boost regimens, may offer the possibility of improved magnitude, breadth, and quality of responses as compared with the homologous rAd5 regimen. Novel antigen concepts, such as centralized consensus[90, 91] and mosaic[92] immunogens, may also

result in increased breadth of cellular immune responses and improved coverage of viral diversity.

Perhaps the most important research focus should be the development of improved Env immunogens to elicit broadly reactive NAbs. Given the scope of this problem, increased basic research regarding the structure, function, and immunogenicity of the Env glycoprotein will be required. Innovative and high-risk ideas should be pursued, and promising approaches should be tested as rapidly as possible in preclinical studies and eventually in clinical studies. Ultimately, it is likely that a combination vaccine consisting of separate vaccine components that elicit T lymphocytes and NAbs will prove optimal. As a result, development of improved T cell based and antibody based vaccine strategies should be pursued in parallel.

To achieve these goals, it will be critical to attract and to retain talented new investigators to the field. Funding programs should therefore be expanded to encourage junior investigators to explore innovative ideas that address critical problems in the field. Given the scientific challenges currently facing the HIV-1 field, increased support and encouragement of fellows and junior faculty should be viewed as a top priority by both senior investigators and funding organizations. It will also be important for industry to continue to participate in the HIV-1 vaccine field, as biotechnology and pharmaceutical companies have critical knowledge and capacities that are not available in academia, government, and non-profit organizations.

A current debate is whether the HIV-1 vaccine field can “withstand” another vaccine efficacy study failure. For HIV-1, the scientific challenges are enormous, and thus so are the risks in testing any new vaccine concept. Clearly, the decision to advance a vaccine candidate into efficacy trials should be highly selective and based on a rigorous and transparent analysis of preclinical and clinical data. However, there is no way to determine whether a potentially promising vaccine candidate will afford protection in humans other than by conducting a clinical efficacy study. Multiple efficacy trials may therefore be required, and many concepts will undoubtedly fail. We should therefore be ready to accept multiple failures of efficacy studies as part of the expected pathway towards the ultimate successful development of a safe and effective HIV-1 vaccine.

Box 1. Challenges in the Development of a Prophylactic HIV-1 Vaccine

- Extensive viral clade and sequence diversity
- Early establishment of latent viral reservoirs
- Immune correlates of protection unclear
- Viral evasion of humoral and cellular immune responses
- Antibody responses typically type-specific
- No method exists to elicit broadly reactive NAbs
- Attenuated viruses unsafe for human use
- Lack of a small animal model
- Little pharmaceutical interest

Box 2. Current HIV Vaccine Strategies

- Traditional Strategies
  - Live attenuated viruses

- Whole killed viruses
- Protein subunits

- Novel Strategies
  - Plasmid DNA vaccines
  - Live recombinant vectors

Box 3. Recommendations for Preclinical Challenge Studies of T Cell Based Vaccines

- Utilize stringent challenge virus (SIVmac239, SIVmac251)
- Design study with adequate power and follow-up time
- Model clinical regimen with vaccine schedule and dose
- Select rhesus monkeys that lack MHC alleles associated with efficient virologic control (Mamu-A*01, B*17, B*08)
- Avoid the use of a homologous Env antigen
- Assess promising vaccine concepts against both homologous and heterologous viral challenges

Acknowledgements

The author would like to thank R. Dolin, N. Letvin, J. Mascola, and J. McElrath for critically reviewing this manuscript. The author acknowledges support from the National Institutes of Health and the Bill & Melinda Gates Foundation.

References

1. Barre-Sinoussi F, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science (New York, NY) 1983;220:868–71.
2. Gallo RC, et al. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science (New York, NY) 1984;224:500–3.
3. Popovic M, Sarngadharan MG, Read E, Gallo RC. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science (New York, NY) 1984;224:497–500.
4. Sarngadharan MG, Popovic M, Bruch L, Schupbach J, Gallo RC. Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS. Science (New York, NY) 1984;224:506–8.
5. Schupbach J, et al. Serological analysis of a subgroup of human T-lymphotropic retroviruses (HTLV-III) associated with AIDS. Science (New York, NY) 1984;224:503–5.
6. Fauci AS. 25 years of HIV. Nature 2008;453:289–90. [PubMed: 18480799]
7. Quinn TC, et al. Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai Project Study Group. N Engl J Med 2000;342:921–9. [PubMed: 10738050]
8. Mascola JR, et al. Immunization with envelope subunit vaccine products elicits neutralizing antibodies against laboratory-adapted but not primary isolates of human immunodeficiency virus type 1. The National Institute of Allergy and Infectious Diseases AIDS Vaccine Evaluation Group. J Infect Dis 1996;173:340–8. [PubMed: 8568294]
9. Moore JP, et al. Primary isolates of human immunodeficiency virus type 1 are relatively resistant to neutralization by monoclonal antibodies to gp120, and their neutralization is not predicted by studies with monomeric gp120. J Virol 1995;69:101–9. [PubMed: 7527081]
10. Flynn NM, et al. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. The Journal of infectious diseases 2005;191:654–65. [PubMed: 15688278]

11. Pitisuttithum P, et al. Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. The Journal of infectious diseases 2006;194:1661–71. [PubMed: 17109337]
12. Priddy FH, et al. Safety and immunogenicity of a replication-incompetent adenovirus type 5 HIV-1 clade B gag/pol/nef vaccine in healthy adults. Clinical infectious diseases 2008;46:1769–81. [PubMed: 18433307]
13. Fauci, A. S. The release of new data from the HVTN 502 (STEP) HIV vaccine study. NIH News, November 7 (2007). These data demonstrate that a homologous rAd5-Gag/Pol/Nef vaccine regimen did not protect against HIV-1 in humans and may have increased the risk of HIV-1 acquisition in individuals with pre-existing Ad5-specific NAbs.
14. Gaschen B, et al. Diversity considerations in HIV-1 vaccine selection. Science 2002;296:2354–60. [PubMed: 12089434]
15. Walker BD, Korber BT. Immune control of HIV: the obstacles of HLA and viral diversity. Nat Immunol 2001;2:473–5. [PubMed: 11376327]
16. Montefiori D, Sattentau Q, Flores J, Esparza J, Mascola J. Antibody-based HIV-1 vaccines: recent developments and future directions. PLoS medicine 2007;4:e348. [PubMed: 18052607]
17. Kwong PD, et al. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. Nature 1998;393:648–59. [PubMed: 9641677]
18. Wyatt R, et al. The antigenic structure of the HIV gp120 envelope glycoprotein. Nature 1998;393:705–11. [PubMed: 9641684]
19. Chen B, et al. Structure of an unliganded simian immunodeficiency virus gp120 core. Nature 2005;433:834–41. [PubMed: 15729334]
20. Wei X, et al. Antibody neutralization and escape by HIV-1. Nature 2003;422:307–12. [PubMed: 12646921]
21. Richman DD, Wrin T, Little SJ, Petropoulos CJ. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. Proceedings of the National Academy of Sciences of the United States of America 2003;100:4144–9. [PubMed: 12644702]
22. Li Y, et al. Broad HIV-1 neutralization mediated by CD4-binding site antibodies. Nature medicine 2007;13:1032–4.
23. Zhou T, et al. Structural definition of a conserved neutralization epitope on HIV-1 gp120. Nature 2007;445:732–7. [PubMed: 17301785]
24. Haynes BF, et al. Cardiolipin polyspecific autoreactivity in two broadly neutralizing HIV-1 antibodies. Science (New York, NY) 2005;308:1906–8.
25. Sun ZY, et al. HIV-1 broadly neutralizing antibody extracts its epitope from a kinked gp41 ectodomain region on the viral membrane. Immunity 2008;28:52–63. [PubMed: 18191596]
26. Frey G, et al. A fusion-intermediate state of HIV-1 gp41 targeted by broadly neutralizing antibodies. Proceedings of the National Academy of Sciences of the United States of America 2008;105:3739–44. [PubMed: 18322015]
27. Baba TW, et al. Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian-human immunodeficiency virus infection. Nature medicine 2000;6:200–6.
28. Mascola JR, et al. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. Nat Med 2000;6:207–10. [PubMed: 10655111]
29. Pantaleo G, et al. Major expansion of CD8+ T cells with a predominant V beta usage during the primary immune response to HIV. Nature 1994;370:463–7. [PubMed: 8047166]
30. Koup RA, et al. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. J Virol 1994;68:4650–5. [PubMed: 8207839]
31. Borrow P, Lewicki H, Hahn BH, Shaw GM, Oldstone MB. Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. J Virol 1994;68:6103–10. [PubMed: 8057491]
32. Musey L, et al. Cytotoxic-T-cell responses, viral load, and disease progression in early human immunodeficiency virus type 1 infection. N Engl J Med 1997;337:1267–74. [PubMed: 9345075]

33. Kiepiela P, et al. Dominant influence of HLA-B in mediating the potential co-evolution of HIV and HLA. Nature 2004;432:769–75. [PubMed: 15592417]
34. Kiepiela P, et al. CD8+ T-cell responses to different HIV proteins have discordant associations with viral load. Nat Med 2007;13:46–53. [PubMed: 17173051]
35. Schmitz JE, et al. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science 1999;283:857–60. [PubMed: 9933172]
36. Jin X, et al. Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. J Exp Med 1999;189:991–8. [PubMed: 10075982]
37. Phillips RE, et al. Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition. Nature 1991;354:453–9. [PubMed: 1721107]
38. Allen TM, et al. Tat-specific cytotoxic T lymphocytes select for SIV escape variants during resolution of primary viraemia. Nature 2000;407:386–90. [PubMed: 11014195]
39. Barouch DH, et al. Eventual AIDS vaccine failure in a rhesus monkey by viral escape from cytotoxic T lymphocytes. Nature 2002;415:335–9. [PubMed: 11797012]
40. Betts MR, et al. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. Blood 2006;107:4781–9. [PubMed: 16467198]
41. Precopio ML, et al. Immunization with vaccinia virus induces polyfunctional and phenotypically distinctive CD8(+) T cell responses. J Exp Med 2007;204:1405–16. [PubMed: 17535971]
42. Darrah PA, et al. Multifunctional TH1 cells define a correlate of vaccine-mediated protection against Leishmania major. Nat Med 2007;13:843–50. [PubMed: 17558415]
43. Watkins DI, Burton DR, Kallas EG, Moore JP, Koff WC. Nonhuman primate models and the failure of the Merck HIV-1 vaccine in humans. Nature medicine 2008;14:617–21.
44. Seder RA, Darrah PA, Roederer M. T-cell quality in memory and protection: implications for vaccine design. Nature reviews 2008;8:247–58.
45. Chun TW, et al. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. Nature 1997;387:183–8. [PubMed: 9144289]
46. Chun TW, et al. Early establishment of a pool of latently infected, resting CD4(+) T cells during primary HIV-1 infection. Proceedings of the National Academy of Sciences of the United States of America 1998;95:8869–73. [PubMed: 9671771]
47. Douek DC, et al. HIV preferentially infects HIV-specific CD4+ T cells. Nature 2002;417:95–8. [PubMed: 11986671]
48. Veazey RS, et al. Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. Science 1998;280:427–31. [PubMed: 9545219]
49. Mattapallil JJ, et al. Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. Nature 2005;434:1093–7. [PubMed: 15793563]
50. Li Q, et al. Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. Nature 2005;434:1148–52. [PubMed: 15793562]
51. Brenchley JM, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nature medicine 2006;12:1365–71.
52. Mattapallil JJ, et al. Vaccination preserves CD4 memory T cells during acute simian immunodeficiency virus challenge. J Exp Med 2006;203:1533–41. [PubMed: 16735692]
53. Daniel MD, Kirchhoff F, Czajak SC, Sehgal PK, Desrosiers RC. Protective effects of a live attenuated SIV vaccine with a deletion in the nef gene. Science 1992;258:1938–41. [PubMed: 1470917]
54. Wyand MS, Manson KH, Garcia-Moll M, Montefiori D, Desrosiers RC. Vaccine protection by a triple deletion mutant of simian immunodeficiency virus. J Virol 1996;70:3724–33. [PubMed: 8648707]
55. Learmont JC, et al. Immunologic and virologic status after 14 to 18 years of infection with an attenuated strain of HIV-1. A report from the Sydney Blood Bank Cohort. N Engl J Med 1999;340:1715–22. [PubMed: 10352163]
56. Baba TW, et al. Pathogenicity of live, attenuated SIV after mucosal infection of neonatal macaques. Science 1995;267:1820–5. [PubMed: 7892606]
57. Baba TW, et al. Live attenuated, multiply deleted simian immunodeficiency virus causes AIDS in infant and adult macaques. Nat Med 1999;5:194–203. [PubMed: 9930868]

58. Murphey-Corb M, et al. A formalin-inactivated whole SIV vaccine confers protection in macaques. Science 1989;246:1293–7. [PubMed: 2555923]
59. Wille-Reece U, et al. HIV Gag protein conjugated to a Toll-like receptor 7/8 agonist improves the magnitude and quality of Th1 and CD8+ T cell responses in nonhuman primates. Proceedings of the National Academy of Sciences of the United States of America 2005;102:15190–4. [PubMed: 16219698]
60. Wille-Reece U, et al. Toll-like receptor agonists influence the magnitude and quality of memory T cell responses after prime-boost immunization in nonhuman primates. The Journal of experimental medicine 2006;203:1249–58. [PubMed: 16636134]
61. Casimiro DR, et al. Comparative immunogenicity in rhesus monkeys of DNA plasmid, recombinant vaccinia virus, and replication-defective adenovirus vectors expressing a human immunodeficiency virus type 1 gag gene. J Virol 2003;77:6305–13. [PubMed: 12743287]
62. Graham BS, et al. Phase 1 Safety and Immunogenicity Evaluation of a Multiclade HIV-1 DNA Candidate Vaccine. J Infect Dis 2006;194:1650–60. [PubMed: 17109336]
63. Barouch DH, et al. Control of viremia and prevention of clinical AIDS in rhesus monkeys by cytokine-augmented DNA vaccination. Science 2000;290:486–92. [PubMed: 11039923]
64. Chong SY, et al. Comparative ability of plasmid IL-12 and IL-15 to enhance cellular and humoral immune responses elicited by a SIVgag plasmid DNA vaccine and alter disease progression following SHIV(89.6P) challenge in rhesus macaques. Vaccine 2007;25:4967–82. [PubMed: 17335943]
65. Luckay A, et al. Effect of plasmid DNA vaccine design and in vivo electroporation on the resulting vaccine-specific immune responses in rhesus macaques. J Virol 2007;81:5257–69. [PubMed: 17329330]
66. Liu J, Kjeken R, Mathiesen I, Barouch DH. Recruitment of antigen-presenting cells to the site of inoculation and augmentation of human immunodeficiency virus type 1 DNA vaccine immunogenicity by in vivo electroporation. Journal of virology 2008;82:5643–9. [PubMed: 18353952]
67. Shiver JW, et al. Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. Nature 2002;415:331–5. [PubMed: 11797011]
68. Catanzaro AT, et al. Phase 1 safety and immunogenicity evaluation of a multiclade HIV-1 candidate vaccine delivered by a replication-defective recombinant adenovirus vector. J Infect Dis 2006;194:1638–49. [PubMed: 17109335]
69. Amara RR, et al. Control of a mucosal challenge and prevention of AIDS by a multiprotein DNA/MVA vaccine. Science 2001;292:69–74. [PubMed: 11393868]
70. Harari A, et al. An HIV-1 clade C DNA prime, NYVAC boost vaccine regimen induces reliable, polyfunctional, and long-lasting T cell responses. The Journal of experimental medicine 2008;205:63–77. [PubMed: 18195071]
71. Shiver JW, Emini EA. Recent advances in the development of HIV-1 vaccines using replication-incompetent adenovirus vectors. Annu Rev Med 2004;55:355–72. [PubMed: 14746526]
72. Casimiro DR, et al. Attenuation of simian immunodeficiency virus SIVmac239 infection by prophylactic immunization with dna and recombinant adenoviral vaccine vectors expressing Gag. J Virol 2005;79:15547–55. [PubMed: 16306625]This manuscript demonstrates that homologous rAd5 vaccine regimens were minimally effective against SIVmac239 challenges in rhesus monkeys
73. Mothe BR, et al. Expression of the major histocompatibility complex class I molecule Mamu-A*01 is associated with control of simian immunodeficiency virus SIVmac239 replication. J Virol 2003;77:2736–40. [PubMed: 12552014]
74. Pal R, et al. ALVAC-SIV-gag-pol-env-based vaccination and macaque major histocompatibility complex class I (A*01) delay simian immunodeficiency virus SIVmac-induced immunodeficiency. J Virol 2002;76:292–302. [PubMed: 11739694]
75. Zhang ZQ, et al. Mamu-A*01 allele-mediated attenuation of disease progression in simian-human immunodeficiency virus infection. J Virol 2002;76:12845–54. [PubMed: 12438610]
76. Wilson NA, et al. Vaccine-induced cellular immune responses reduce plasma viral concentrations after repeated low-dose challenge with pathogenic simian immunodeficiency virus SIVmac239. J Virol 2006;80:5875–85. [PubMed: 16731926]

77. Letvin NL, et al. Preserved CD4+ central memory T cells and survival in vaccinated SIV-challenged monkeys. Science 2006;312:1530–3. [PubMed: 16763152]

78. Vogels R, et al. Replication-deficient human adenovirus type 35 vectors for gene transfer and vaccination: efficient human cell infection and bypass of preexisting adenovirus immunity. J Virol 2003;77:8263–71. [PubMed: 12857895]

79. Abbink P, et al. Comparative seroprevalence and immunogenicity of six rare serotype recombinant adenovirus vaccine vectors from subgroups B and D. J Virol 2007;81:4654–63. [PubMed: 17329340]

80. Thorner AR, et al. Age dependence of adenovirus-specific neutralizing antibody titers in individuals from sub-Saharan Africa. J Clin Microbiol 2006;44:3781–3. [PubMed: 17021110]

81. Kostense S, et al. Adenovirus types 5 and 35 seroprevalence in AIDS risk groups supports type 35 as a vaccine vector. Aids 2004;18:1213–6. [PubMed: 15166541]

82. Fauci AS, et al. HIV vaccine research: the way forward. Science (New York, NY) 2008;321:530–2.This perspective describes revised NIAID research priorities for HIV-1 vaccine research

83. Catanzaro AT, et al. Phase I clinical evaluation of a six-plasmid multiclade HIV-1 DNA candidate vaccine. Vaccine 2007;25:4085–92. [PubMed: 17391815]

84. Fauci AS. NIAID will not move forward with the PAVE 100 HIV vaccine trial. NIH News. July 17;2008

85. Barouch DH, et al. Immunogenicity of recombinant adenovirus serotype 35 vaccine in the presence of pre-existing anti-Ad5 immunity. J Immunol 2004;172:6290–7. [PubMed: 15128818]

86. Roberts DM, et al. Hexon-chimaeric adenovirus serotype 5 vectors circumvent pre-existing anti-vector immunity. Nature 2006;441:239–43. [PubMed: 16625206]

87. Farina SF, et al. Replication-defective vector based on a chimpanzee adenovirus. J Virol 2001;75:11603–13. [PubMed: 11689642]

88. Fitzgerald JC, et al. A simian replication-defective adenoviral recombinant vaccine to HIV-1 gag. J Immunol 2003;170:1416–22. [PubMed: 12538702]

89. Liu J, et al. Magnitude and phenotype of cellular immune responses elicited by recombinant adenovirus vectors and heterologous prime-boost regimens in rhesus monkeys. Journal of virology 2008;82:4844–52. [PubMed: 18337575]

90. Liao HX, et al. A group M consensus envelope glycoprotein induces antibodies that neutralize subsets of subtype B and C HIV-1 primary viruses. Virology 2006;353:268–82. [PubMed: 17039602]

91. Weaver EA, et al. Cross-subtype T-cell immune responses induced by a human immunodeficiency virus type 1 group m consensus env immunogen. J Virol 2006;80:6745–56. [PubMed: 16809280]

92. Fischer W, et al. Polyvalent vaccines for optimal coverage of potential T-cell epitopes in global HIV-1 variants. Nat Med 2007;13:100–6. [PubMed: 17187074]This manuscript proposes the use of polyvalent “mosaic” antigens to improve immunologic coverage of global HIV-1 diversity

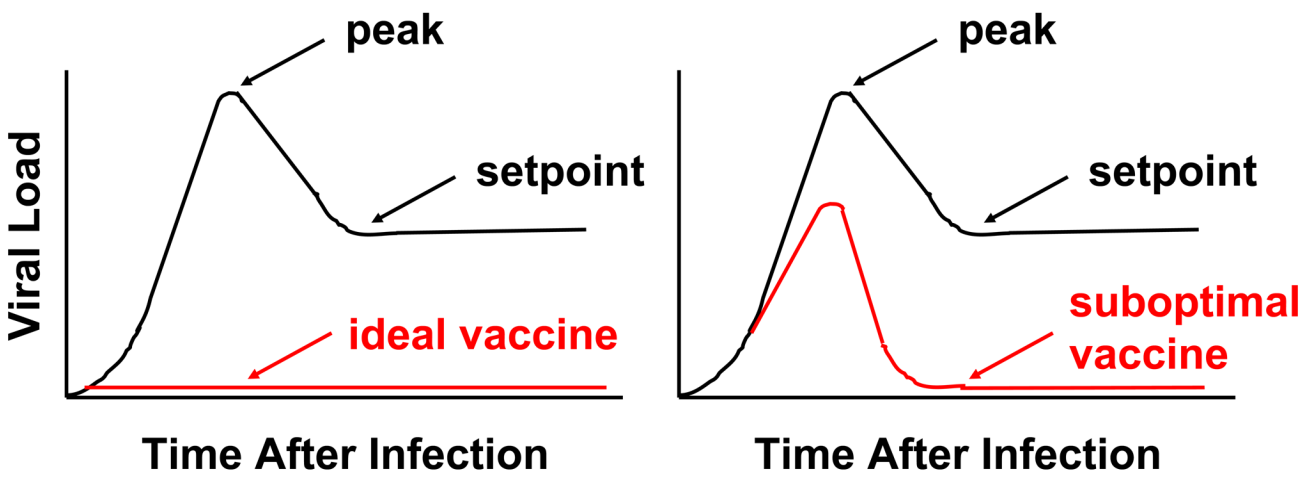

Figure 1. Goals of an HIV-1 vaccine

Following infection, HIV-1 replicates exponentially to a peak level and then is partially controlled to a viral setpoint level (black). Left, an ideal vaccine would protect against infection and afford sterilizing immunity (red). Right, a suboptimal vaccine would result in decreased peak and setpoint viral loads following infection (red).

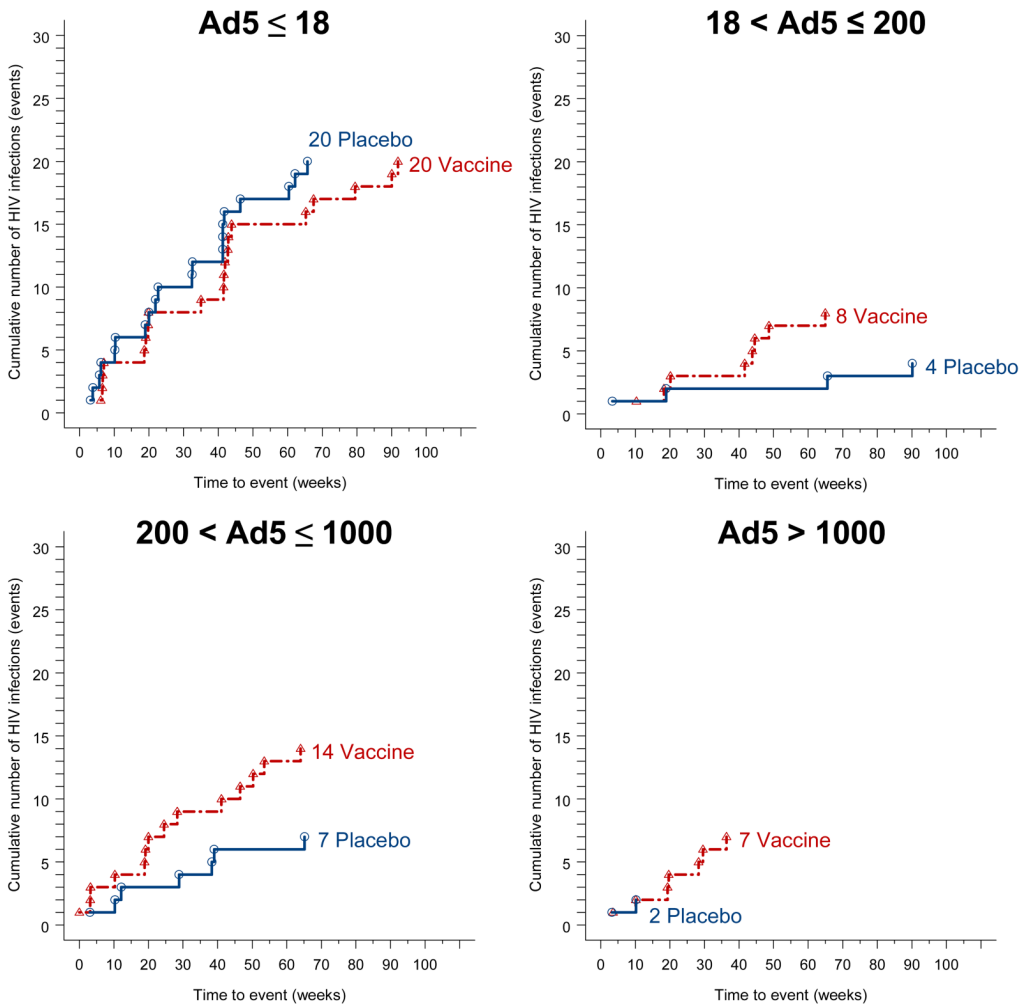

Figure 2. Cumulative HIV-1 infections in men enrolled in the STEP study as stratified by pre-existing Ad5-specific NAb titer

Cumulative infections as of October 17, 2007 in men enrolled in the STEP study (HVTN 502) evaluating the Merck rAd5-Gag/Pol/Nef vaccine are depicted. Infections in vaccinees (red) and placebos (blue) are shown in individuals as stratified by pre-existing Ad5-specific NAb titers. Data represent the modified intent-to-treat population. Courtesy of Dr. Michael Robertson, Merck Research Laboratories.