REVIEWS

Challenges in the development of an HIV-1 vaccine

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The development of a safe and effective human immunodeficiency virus (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 so far failed to protect against HIV-1 infection or to reduce viral loads after 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]. More than 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 after 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 most clinically licensed vaccines. In contrast, most licensed viral vaccines seem 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 after infection. Such partial control, as exemplified by a reduction in peak and setpoint viral loads after infection, has been demonstrated in certain preclinical studies by vaccines that elicit T lymphocyte responses.
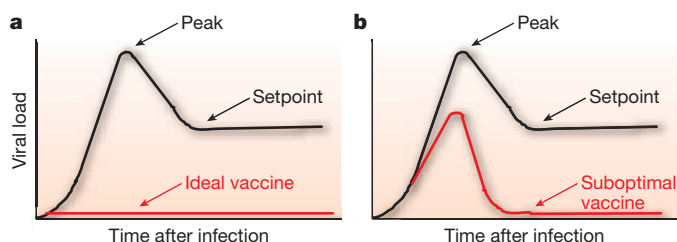
Moreover, because viral loads represent a principal determinant of HIV-1 transmission[7], it is conceivable that such a partially protective vaccine might have substantial impact on a population level.

Despite the urgent need for an HIV-1 vaccine, only two vaccine concepts have completed clinical efficacy studies so far. The first vaccine concept used monomeric HIV-1 Env gp120 protein, 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[8,9]. In 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 most subjects, although these responses were partially suppressed in individuals with pre-existing Ad5-specific neutralizing antibodies[12]. Phase 2b efficacy trials sponsored by Merck and the National Institutes of Health (NIH) were unexpectedly terminated when the first planned interim analysis showed that this vaccine failed to protect against infection or to reduce viral loads after infection, and that vaccinees with pre-existing Ad5-specific neutralizing antibodies 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

Figure 1 | Goals of an HIV-1 vaccine. After infection, HIV-1 replicates exponentially to a peak level and then is partially controlled to a viral setpoint level (black). **a**, An ideal vaccine would protect against infection and afford sterilizing immunity (red). **b**, A suboptimal vaccine would result in decreased peak and setpoint viral loads after infection (red).

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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, because 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 non-human 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 probably only emerge in the context of successful vaccine efficacy studies in humans.

HIV-1-specific humoral immunity. Virus-specific neutralizing antibody titres represent key immune correlates of protection 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 neutralizing antibodies 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 binding site of the chemokine co-receptor, 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 neutralizing antibody responses[20,21].

Nevertheless, broadly reactive neutralizing antibody activity has been identified in a small number of HIV-1-infected subjects, and this reactivity seems to be largely directed against conserved regions of the Env glycoprotein such as the CD4-binding site[22]. The broadly reactive monoclonal antibody 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 monoclonal antibodies 2F5 and 4E10. However, MPER-specific neutralizing antibodies 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 neutralizing antibodies is perhaps the most important priority for the HIV-1 vaccine field[16]. Proof-of-concept passive transfer studies in non-human primates have shown that administration of high doses of broadly reactive monoclonal antibodies 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 neutralizing antibodies by vaccination so far. 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 neutralizing antibodies 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 have 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 non-progressors[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+ 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 by 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 interferon-γ secretion as measured by enzyme-linked immunospot (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 non-progressors[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 probably not protect against acquisition of HIV-1 infection. As a result, vaccine-induced T lymphocyte responses will presumably be unable to prevent lifelong infection, because 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 most memory CD4$^+$ T lymphocytes in gut-associated lymphoid tissue 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 probably 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 after 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 they are unlikely to be used in humans owing 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 neutralizing antibody responses as well as by their inability to elicit CD8$^+$ T lymphocyte responses. Recent data, however, suggest that Toll-like receptor adjuvants may increase the utility of protein subunit immunogens[59,60].

Novel strategies. New 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 non-human 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 most HIV-1 vaccine candidates that are currently in clinical trials. Other viral vectors that are being evaluated include vesicular stomatitis virus, adeno-associated virus, Venezuelan equine encephalitis virus, cytomegalovirus, herpes simplex virus and measles virus. Bacterial and mycobacterial vectors are also being explored, including *Salmonella*, *Listeria* and Bacille Calmette-Guérin (BCG).

The STEP study

Preclinical background. Recombinant Ad5 vectors were selected for development by Merck on the basis of 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 marked reductions of viral loads after challenge of rhesus monkeys with the chimaeric simian–human immunodeficiency virus (SHIV)-89.6P (ref. 67). However, it was also observed that the same vaccine afforded minimal to no control of peak or setpoint viral loads after challenge with SIV$_{MAC239}$ (ref. 72), indicating that SIV challenges were considerably more stringent than SHIV-89.6P challenges.

Box 2 | Current HIV vaccine strategies

(1) Traditional strategies
      Live attenuated viruses
      Whole killed viruses
      Protein subunits
(2) Novel strategies
      Plasmid DNA vaccines
      Live recombinant vectors

A DNA prime/rAd5 boost regimen expressing SIV Gag afforded a brief (90 days) and marginal (0.8 log) reduction of peak viral loads after SIV$_{MAC239}$ challenge[72], but this effect was only observed in rhesus monkeys that were selected to express the major histocompatibility complex (MHC) class I molecule Mamu-A*01, which 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 been able to reduce setpoint viral loads after SIV challenge of Mamu-A*01-negative rhesus monkeys so far[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 most volunteers[12]. However, as predicted by preclinical studies[61], responses to this vaccine were partially suppressed in individuals with pre-existing neutralizing antibodies against the vaccine vector. Because 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 neutralizing antibodies[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 after infection. HIV Vaccine Trials Network (HVTN) 502, also known as the 'STEP' study, was a 3,000-subject study in the Americas, the Caribbean 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 18 September 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 end points[13]. Moreover, in subjects with pre-existing Ad5-specific neutralizing antibody titres, a greater number of HIV-1 infections occurred in vaccinees than in 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-specific neutralizing antibodies 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 neutralizing antibodies was unexpected, and this finding highlights our lack of understanding of the parameters that determine susceptibility to HIV-1 infection. The biological basis for this observation remains unclear. One hypothesis is that rAd5 vaccination of individuals with pre-existing Ad5-specific neutralizing antibodies may have resulted in potent anamnestic Ad5-specific CD4$^+$ T lymphocytes that were increased targets for HIV-1 infection. However, early data have suggested that Ad5-specific T lymphocyte responses after rAd5 vaccination are actually lower in individuals with pre-existing Ad5-specific neutralizing antibodies than in those without pre-existing Ad5-specific neutralizing antibodies (J. McElrath,
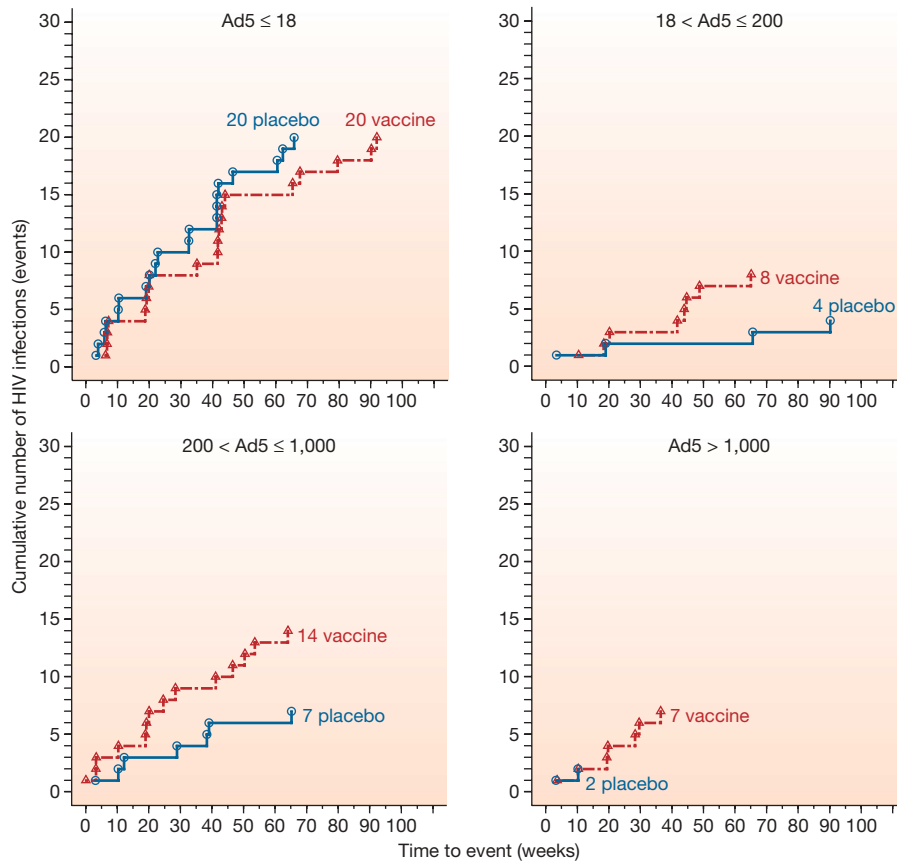
Figure 2 | Cumulative HIV-1 infections in men enrolled in the STEP study stratified by pre-existing Ad5-specific neutralizing antibody titre. Cumulative infections as of 17 October 2007 in men enrolled in the STEP study (HVTN 502) evaluating the Merck rAd5-Gag/Pol/Nef vaccine are depicted. Infections in vaccinees (red) and placebos (blue) are shown in individuals stratified by their pre-existing Ad5-specific neutralizing antibody titres. Data represent the modified intent-to-treat population. Image courtesy of M. Robertson, Merck Research Laboratories.

unpublished observations). An alternative hypothesis is that Ad5-specific neutralizing antibodies may have opsonized rAd5 vectors after immunization, resulting in altered tropism or inflammatory responses. It is also possible that pre-existing Ad5-specific neutralizing antibodies 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 neutralizing antibodies to the vaccine vector that is used 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, non-human primate challenge models should be recalibrated on the basis of the STEP study to guide future HIV-1 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 use SIV$_{MAC239}$ or SIV$_{MAC251}$ 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 physiological 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, because infecting viruses in humans will almost certainly be heterologous to any vaccine sequence. Because very few heterologous SIV challenge studies have been completed so far, a practical approach may be to determine the protective efficacy of promising vaccine

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Box 3 | Recommendations for preclinical challenge studies of T-cell-based vaccines

(1) Use stringent challenge virus (SIV$_{MAC239}$, SIV$_{MAC251}$).
(2) Design study with adequate power and follow-up time.
(3) Model clinical regimen with vaccine schedule and dose.
(4) Select rhesus monkeys that lack MHC alleles associated with efficient virologic control (Mamu-A*01, Mamu-B*17, Mamu-B*08).
(5) Avoid the use of a homologous Env antigen.
(6) Assess promising vaccine concepts against both homologous and heterologous viral challenges.

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candidates against both homologous and heterologous SIV challenges. It is currently debated whether non-human primate challenge studies should be used as a formal 'gatekeeper' for advancing vaccine candidates into clinical efficacy studies, because 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 the development of vaccine candidates that lead to durable control of setpoint viral loads after SIV_{MAC239} or SIV_{MAC251} challenge.

The STEP study has also had a major impact on other HIV-1 vaccine programmes in the field. HVTN 503 was terminated as it used the same rAd5-based vaccine candidate that was used 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 most 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 after a homologous SIV_{MAC251} challenge[77]. However, no durable control of setpoint viral loads was observed with this vaccine, although delayed progression to AIDS-related mortality was evident[77]. NIH 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 using modified vaccinia Ankara (MVA)[69] and NYVAC[70] vectors, and phase 1 clinical trials have demonstrated immunogenicity in most volunteers. Central to all of these programmes, however, is the hypothesis that DNA priming before 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.

New 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 neutralizing antibodies. 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 neutralizing antibodies. 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]; chimaeric rAd5HVR48 vectors in which dominant Ad5-specific neutralizing antibody 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 have been shown to 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 using serologically distinct rAd vectors. We have recently demonstrated that a heterologous rAd26 prime/Ad5 boost regimen expressing SIV Gag afforded a durable 2.4 log reduction of setpoint viral loads after SIV_{MAC251} challenge of Mamu-A*01-negative rhesus monkeys, whereas a homologous rAd5 regimen provided no protection in this stringent challenge model[90]. 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 T lymphocyte responses as compared with the homologous rAd5 regimen. New antigen concepts, such as centralized consensus[91,92] and mosaic[93] 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 neutralizing antibodies. 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 trials. Ultimately, it is likely that a combination vaccine consisting of separate vaccine components that elicit T lymphocytes and neutralizing antibodies 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 programmes 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 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.

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