Published in final edited form as:

Curr Opin HIV AIDS. 2010 September ; 5(5): 428–434. doi:10.1097/COH.0b013e32833d17ac.

HIV Vaccines - Lessons learned and the way forward

Jerome H. Kim¹, Supachai Rerks-Ngarm², Jean-Louis Excler³, and Nelson L. Michael¹

¹Walter Reed Army Institute of Research, U.S. Military HIV Research Program (MHRP), Rockville MD, USA ²Department of Disease Control, Ministry of Public Health, Nonthaburi, Thailand ³U.S. Military HIV Research Program (MHRP), Henry M. Jackson Foundation, Rockville MD, USA

Abstract

Purpose of Review—An effective HIV vaccine is a global health priority. We describe lessons learned from four HIV vaccine trials that failed to demonstrate efficacy and one that showed modest protection as a pathway forward.

Recent findings—The Merck Ad5 phase IIb T-cell vaccine failed to show efficacy and might have increased the risk of HIV acquisition in MSM. While VaxGen gp120 alone was not efficacious in groups at high risk for HIV-1 infection, the RV144 ALVAC prime and gp120 boost regimen showed 31% efficacy in low incidence heterosexuals. All trials demonstrated the limitations of available laboratory and animal models to both assess relevant vaccine-induced immune responses and to predict clinical trial outcome. Analysis of innate and adaptive responses induced in RV 144 will guide future trial design.

Summary—Future HIV vaccine trials should define the RV 144 immune responses relevant to protection, improve durability and level of protection, and assess efficacy in diverse risk groups. New strategies examining heterologous vector prime boost, universal inserts, replicating vectors, and novel protein/adjuvant immunogens should be explored to induce both T-cell and antibody responses. HIV vaccine development requires innovative ideas and a sustained long-term commitment of scientists, governments, and the community.

Keywords

HIV; vaccine; Thailand; clinical trial; efficacy

Introduction

In 2008, the total number of people living with HIV was estimated to be 33.4 million people, 97% living in low- and mid-income countries [1]. The development of a safe and efficacious preventive HIV vaccine, as part of a comprehensive prevention program, remains among the highest global health priorities and the best long-term tool for the control of the HIV -1.

The development of a preventive HIV vaccine faces unprecedented scientific and obstacles (Table 1) [2-5]. The immune correlates, quality and magnitude of the immune responses needed to confer protection against HIV infection are still unclear.

Corresponding author: Jerome H. Kim, MD, Walter Reed Army Institute of Research, U.S. Military HIV Research Program (MHRP), 1600 E. Gude Drive, Rockville, MD 20850 USA, Tel: 301-251-7764, Fax: 301-762-7460, Jkim@hivresearch.org.

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HIV envelope proteins failed to protect high-risk volunteers in two efficacy trials [6,7*]. Current AIDS vaccine candidates are unable to induce broadly neutralizing antibodies (bNAb) against primary HIV isolates or only to a very limited and narrow extent [8], representing a major stumbling block in the development of an effective HIV vaccine [9].

The immune response elicited by a successful vaccine likely will require both antibodies and T cells that recognize, neutralize and/or inactivate diverse strains of HIV and that reach the site of infection before the infection becomes irreversibly established [10]. Given the hurdles of eliciting bNAb, the focus of HIV vaccine development turned to evaluating vaccines capable of reducing viral replication after infection (“T-cell vaccines”). The control of viral replication could conceivably slow the rate of disease progression as suggested by non-human primate (NHP) challenge studies [11-14*] and/or reduce transmission of HIV from infected vaccinee to partner [15].

The goal of a vaccination regimen designed to induce cell-mediated immune (CMI) responses should therefore be to reduce the plasma viral load at set point and preserve memory CD4+ lymphocytes. Clinical efforts have mainly focused on CMI-inducing vaccines such as DNA and vectors alone or in prime-boost regimens [16,17] (Table 2). In humans, the response to HIV genes inserted into viral vectors with or without DNA priming have been variable in frequency, magnitude and breadth [18-22]. Such regimens at various degrees have conferred protection, defined as reduced setpoint viral load, in the SHIV and SIV challenge macaque models [23,24].

The Step (HVTN 502/Merck 023) and Phambili (HVTN 503) vaccine trials were the first human efficacy trials (phase IIb ‘test-of-concept’) to explore whether an HIV-1 prophylactic vaccine aimed at inducing CMI responses could prevent infection or reduce post-infection viremia. The Merck vaccine was composed of replication-incompetent adenovirus serotype 5 (MRKAd5 HIV-1) vectors expressing HIV-1 clade B antigens. The Step study enrolled subjects, predominantly high-risk populations including men who have sex with men (MSM) as well as heterosexual women in North and South America and Australia, and heterosexual women and men in the Caribbean [25*,26]. The Phambili study enrolled heterosexual men and women in South Africa [27]. HVTN 502 was unexpectedly halted for futility in achieving the study primary endpoints (follow-up continued for two years after interim analysis), virtually all of which were in MSM. 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. The biological basis for this observation remains unclear. 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 [28].

Although MRKAd5 HIV-1 vaccine failed to show efficacy, the trial demonstrated that the intravenous SHIV NHP challenge model with existing SHIVs is misleading and inadequate for evaluating T-cell vaccines. While the SIV challenge model better predicted the observed failure to reduce viral load setpoint, it failed to explain acquisition outcomes [14]. Other conclusions were that immunity to vectors, including at the tissue level, should be evaluated in future clinical studies [18] and that smaller efficacy trial designs might yield valuable information to guide future efforts [29]. The design of parallel NHP and clinical studies for a more direct comparison of the results would help identify and validate NHP models. A DNA and Ad5 prime-boost regimen is being tested in a Phase IIb trial (HVTN 505) in circumcised, Ad5-specific serum antibody negative, high-risk MSM in the United States [30].

The potentially misleading nature of the current laboratory-based measures of immunogenicity and the relevance of those responses in the prevention of HIV acquisition were highlighted when the results of a phase IIb trial (RV144) of ALVAC-HIV and AIDSVAX® gp120 B/E

prime-boost were announced [31**]. AIDSVAX® gp120 B/E showed no efficacy in a Phase III trial in Thai injecting drug users [7]. RV144 enrolled Thai volunteers at “community risk” for HIV infection. Although the trial had been criticized scientifically [32], RV144 showed that, by modified intent-to-treat analysis 3.5 years after initial vaccination, the vaccine regimen was 31.2 % efficacious in preventing HIV infection. There was no effect on early post-infection HIV-1 RNA viral load or CD4+ T-cell count. Interestingly, a simple, combined analysis of previous phase I and II ALVAC-HIV and gp120 prime-boost studies showed a rate of HIV-1 infection of 0.59 per 100 person-years in the vaccine group and 1.2 per 100 person-years in the placebo group, for a vaccine efficacy (VE) of 50%, a difference that was not statistically significant; the results also showed no effect on viral load as well [33].

The post-RV144 way forward

While the level of efficacy observed in RV144 is modest, it represents a major step forward for HIV vaccines, providing the first evidence that a safe and effective preventive HIV vaccine is possible. Two findings that did not achieve statistical significance in the pre-specified analysis may be the basis for future hypothesis testing. The first was the non-statistically significant observation, that when stratifying for baseline risk, those with the lowest risk (yearly incidence: 0.23/100 person years) had an apparent efficacy of 40%, whereas those with the highest baseline (incidence 0.36/100 person-years) had efficacy of 3.7%. The second important observation was that VE appeared to decrease with time; at month 12 of the study it was 60% (using a Kaplan Meier estimate) and fell to 29% by month 42 (Kaplan Meier estimate). If early VE of 50% could be extended with boosting, it would have important implications for vaccine development.

Whether the observed efficacy is the result of the prime-boost regimen or of one of its components is unknown. An immunologic correlate with protection from HIV-1 infection has not been determined. RV144 HIV-infected vaccine and placebo recipients are currently being evaluated in a prospective cohort study assessing the difference in a composite endpoint (time to CD4 count <350 or antiretroviral treatment or AIDS-related illness). Additional studies of more recently developed immunogenicity assays are planned in order to determine their suitability for correlates analyses. Further insight may be gained through molecular-sieve analysis of breakthrough infections with the use of single-genome amplification, as the use of this technique in the Step trial has revealed changes in HIV sequences (albeit without an effect on viral load) likely due to vaccine-induced immune pressure [34,35].

Another fundamental question is whether the trend in higher, early efficacy observed in RV144 could be sustained and improved by booster vaccinations (Table 2). To address this question, a first study will assess the residual vaccine-induced memory immune responses in RV144 vaccine recipients through the administration of booster injections of ALVAC-HIV, AIDSVAX B/E or the combination. A second study plans to explore in depth and at earlier time points the immune responses induced by the prime-boost regimen and (12-month) booster vaccinations. Humoral, innate and adaptive cell-mediated mucosal immune responses deserve special consideration as well as innovative explorations such as the signatures of innate immunity and early host responses to vaccination [36-38] and transport of HIV-1 in cervico-vaginal secretions [39].

If efforts to identify a correlate of protection fail, a rational approach to the advanced clinical development of the ALVAC-HIV and AIDSVAX® gp120 B/E prime-boost regimen would confirm and extend the findings in RV144 by further efficacy testing under scientifically parsimonious conditions (Table 2). This approach focuses on two hypotheses: 1) the use of a booster dose at 12 months will extend the durability of protection seen at 12 months; and 2) the use of a booster injection will demonstrate an effect in higher risk volunteers. The ALVAC-HIV and AIDSVAX® gp120 B/E prime-boost regimen with an additional booster injection at

12 months could be tested in a large community risk Thai population maintaining the RV 144 population profile for risk, HIV incidence and subtype distribution, and vaccine products thus minimizing confounding variables. This vaccination strategy would provide potential proof-of-concept that extension of the observed 60% efficacy from 12 months to 24 months was possible. Evaluation of the RV144 regimen with a boost in Thai MSM would vary the route of transmission in a population with higher HIV incidence and increased risk for HIV infection [40*], but other potential variables mentioned are kept constant. The study may provide evidence that the RV144 regimen might be modified with a booster vaccination by increasing and sustaining the immune responses (as suggested by the RV144 waning efficacy over time) and possibly protect persons at higher risk of infection, populations still responsible for a large number of new infections in Thailand today [41,42]. Appropriately designed specimen collection would maximize the opportunity to identify potential correlates by collecting more frequent and larger sample volumes than were obtained in RV144. Finally, testing subtype C cognate immunogens in high-incidence heterosexuals at risk for subtype C infection in southern Africa would blend the value of testing the proof-of-concept demonstrated in RV144 in higher incidence populations with a different, albeit homogeneous, HIV-1 subtype, host genetic and environmental background, but by a similar route of infection. The demonstration of an effective HIV vaccine in high incidence populations would be of great public health relevance.

Issues and innovative approaches in vaccine design and clinical testing

The modest success of RV144 should inform, but not supplant, other work towards the development of an HIV vaccine. In the following section we identify issues common to all vaccine approaches and place them within the context of what we have learned to date in RV144.

Correlates of protection and tests of immunogenicity

The Step and RV144 efficacy trials illustrate the limits and need to revisit the concepts for immune protection and the laboratory assays available for assessment of vaccine immunogenicity [43,44]. While the MRKAd5 HIV-1 vaccine induced IFN- γ ELISPOT responses in a majority of vaccine recipients, it did not confer protection from HIV acquisition or reduce in post-infection setpoint viral load. In contrast the RV144 prime-boost regimen conferred a modest efficacy for HIV acquisition without affecting HIV viral load while IFN- γ ELISPOT responses were elicited in less than 20% of vaccine recipients. ELISPOT assays and intracellular cytokine analysis should no longer be the only tools used assess vaccine potency. The development and validation of additional assays measuring lymphoproliferation, mucosal responses [45], cytotoxic capacity, *in-vitro* viral inhibition [46-48], or other immune functions such non-neutralizing antibody avidity [49] and antibody-dependent cytotoxicity and antibody-dependent cell-mediated viral inhibition [50,51] may provide a more robust indication of functional antiviral activity. A particular consideration should be given to the exploration of the vaccine-induced mucosal responses, since a study of ALVAC-SIV with a low-dose, repetitive SIV challenge delivered in milk showed protection of neonatal macaques in the absence of detectable IFN- γ ELISPOT responses; conversely, MVA-SIV did not protect against this challenge despite generating IFN- γ ELISPOT responses [52]. In the absence of a defined correlate of protection, it will be difficult to screen candidates for future clinical development without efficacy testing in phase IIb trials. Several questions arise: 1) can pre-defined immune selection criteria to rank vaccine approaches be applied generally (whatever the approach), within vector platform classes (pox viruses) only, or within insert classes (mosaics, conserved sequences); 2) is more, better, if we do not know what “more” means or should we assume that, like the tide, higher general immune responses will improve the unknown specific response desired?

Animal models

Post Step, work was focused on high-dose intravenous pathogenic SIV challenge since the MRKAd5 HIV-1 vaccine did not have an effect on SIV acquisition or viral load in this challenge model [14] corroborating the Step findings [25]. The modest success of the ALVAC-HIV and AIDSVAX® gp120 B/E prime-boost regimen has focused greater attention on low-dose mucosal challenges in NHP. Recent work suggests that protection against homologous and heterologous infection acquisition is possible, and unlike high dose intravenous challenge, protection against low-dose challenge is not necessarily associated with viral load control in breakthrough infections (Franchini G; Nabel G, Letvin N, personal communication). Despite criticism of the SHIV challenge model since the Step study [14] the development of pathogenic SHIVs that can be used in low-dose challenge settings may be an important part of the development of NHP models that will inform Env subunit design and selection [53].

Induction of broad cell-mediated responses

The Step and RV144 results reinforce the need to develop new vaccines able to induce stronger, broader and more sustained humoral and CMI responses, in particular at the mucosal level [54*].

Heterologous vectors and inserts—If a vaccine can induce greater breadth of T- and B-cell responses to HIV than occurs naturally during acute infection, then the use of a combination of protective epitopes in a preventive vaccine may control the early dissemination of HIV, resulting in a lower viral set point and better long-term immune control [55]. A heterologous regimen with adenovirus prime and poxvirus boost offers promising leads [56-58]. Regimens heterologous for their antigens may broaden the CMI responses against conserved epitopes [59,60,61*,62] as suggested by a regimen with DNA and MVA vaccines heterologous for their inserts inducing strong T-cell responses [63]. Other pox vectors such as MVA and NYVAC in heterologous prime-boost regimens with DNA or vector prime and with or without subunit boost may be tested in future efficacy studies [17,21,64]. Polyvalent ‘mosaic’ antigens are designed to optimize cellular immunologic coverage of global HIV-1 sequence diversity. Mosaic HIV antigens expressed by Ad26 vectors markedly augmented both the breadth and depth of antigen-specific CMI responses as compared with consensus or natural sequence HIV antigens in rhesus monkeys. Polyvalent mosaic and conserved sequence antigens therefore represent promising strategies to expand CMI vaccine coverage of HIV diversity [65*,66].

Replicating Vector Approaches—The development of replicating vectors has triggered increased interest, with the hope they could mimic the efficacy of a live-attenuated SIV vaccine [67] while minimizing the safety concerns, and induce HIV-specific immune responses at the virus mucosal entry point (Table 2) [68,69]. Novel vector approaches may be limited by pre-existing anti-vector immune responses that may blunt vaccine-induced HIV-specific responses [18,70]. Whether pre-existing immunity can be overcome by vector replication or by the use of prime-boost regimens deserves further investigation. Safety concerns for the use of these vectors in humans will represent a significant challenge to clinical development.

Broadly neutralizing antibody—Immunogens that elicit bNAb remain a high priority. All of the known bNAb while providing protection in primate models [71] recognize conserved recessed viral epitopes and failed to elicit bNAb responses when incorporated into immunogens [72,73]. New bNAb recently identified from chronically HIV-infected donors may help HIV vaccine design [74,75*]. Long-lasting serum neutralizing activity has been induced by vaccination in monkeys with a gene transfer viral vector expressing antibodies conferring complete protection against intravenous SIV challenge [76].

Populations, incidence, subtypes, sexually transmitted diseases, host genetics

RV144 was a community-based efficacy trial that did not intend to study the efficacy by risk behavior stratification. A fundamental question is whether the prime-boost regimen would be efficacious in populations with different genetic and environmental background and risks for HIV acquisition. Future efficacy trials should investigate this question in populations with higher HIV prevalence and incidence, in particular heterosexual populations in Africa and South East Asia and in higher risk groups such as female sex workers and MSM with better defined and quantified risk behavior [77,78]. Although the number of endpoints in RV144 does not permit definitive conclusions, post-hoc subgroup analyses suggest that efficacy in the first 12 months was higher in low and moderate risk groups than in the highest risk group; importantly efficacy was seen in the highest risk group at 12 months. New trials should be powered to measure efficacy at earlier time points after vaccination than in RV144. Several hypotheses could be formulated, variables controlled and the trial designed to extend the result observed in RV144. For example, ALVAC-HIV (vCP1521) is a B/E hybrid; would the same efficacy results be observed in an HIV-1 subtype C geographical setting using an ALVAC-HIV subtype C construct? The AIDSVAX® gp120 B/E has a gD (HSV) tag; would an untagged gp120 confer a different structure and quality of the envelope subunit while being equally effective? The rates of HIV incidence in RV144 were low (0.27/100 person years); would a similar result be obtained in a population with a significantly higher incidence? Would VE rates differ when stratified by frequency of sexual intercourse (repeated ‘natural challenge’) over time? Would the clinical trial be comparable if the rates of ulcerative genital disease, known to affect the per coital act risk of transmission and to release the genetic bottleneck to transmission [77,79], were different? Finally, could known or unknown genetic polymorphisms affect HIV acquisition and could these be controlled across populations? The interaction between region-specific HLA genotypes and HIV-1 is only now being analyzed in cohorts other than the RV144; preliminary evidence from these studies suggests that there may be HLA and KIR genotypes that may influence vaccine response and disease progression (Robert Paris, personal communication) [80-82].

Impact of other prevention strategies—With reduced HIV incidence rates due to scale up of prevention strategies, vaccine efficacy trials are multicenter and/or multi-country in order to reach the sample size and endpoints required. Incidence cohort studies should therefore be expanded to meet the need for efficacy testing of new vaccine candidates in the pipeline [83-85]. HIV vaccine and other new prevention technologies and strategies such as pre-exposure prophylaxis, microbicides and antiretroviral treatment for prevention may deserve studies exploring their possible synergistic effect [86,87].

Conclusion

Despite the obstacles that HIV presents to vaccine researchers, the historic success of vaccines for other pathogens argues that HIV vaccine research must be continued and accelerated [88]. The recent results of the RV144 efficacy trial open promising avenues and will boost the efforts of the scientific community. These efforts will require a vibrant clinical research infrastructure linked to innovative discovery research that should be part of new clinical studies. It will also require innovative ideas and a sustained long-term commitment of the scientific community, civil society, politicians and donors and study participants.

Acknowledgments

We are extremely grateful to the HIV vaccine trial volunteers and their supporting communities whose willing participation in vaccine clinical trials has greatly advanced the field.

These studies were supported in part by an Interagency Agreement Y1-AI-2642-12 between U.S. Army Medical Research and Materiel Command (USAMRMC) and the National Institutes of Allergy and Infectious Diseases. In addition this work was supported by a cooperative agreement (W81XWH-07-2-0067) between the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., and the U.S. Department of Defense (DOD).

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Table 1
Scientific and non-scientific obstacles to the development of an HIV vaccine

- Extensive viral subtype and sequence diversity limiting efficacy of current vaccine approaches to specific subtypes
- Narrow window of opportunity for the immune system to clear initial infection before early establishment of latent viral reservoirs
- Immune correlates of protection remain unknown
- Viral escape from humoral and cellular immune responses may limit sustained efficacy
- Conserved antibody targets on the outer envelope protein are hidden
- Lack of a predictive animal model
- Limited interest of the pharmaceutical industry
- Limited long term sustained commitment from governments and donors

Table 2
Proposed post RV144 clinical development pathway and priorities

Short-term strategy <i>Understanding what happened, identifying a correlate, and improving the vaccine</i>	Long-term strategy <i>Advancing the result, optimizing the regimen for other populations</i>
RV144 laboratory studies to identify an immune correlate of protection	
Late boosting study of RV144 vaccinated volunteers	Phase IIB efficacy study of RV144 regimen in a clade E (Thai) (high vs. low incidence), heterosexual vs. men who have sex with men populations.
Immunogenicity study reproducing the RV144 regimen with different boosting options	Phase IIB efficacy study in a non-clade E, higher-incidence population (clade-matched antigens)
Improvements and additional Phase I exploratory studies: ALVAC, other pox vector such as NYVAC and possible new boost, insert, adjuvant; heterologous prime-boost regimens with new vectors and inserts (adenovirus, MVA, mosaics)	
Cohort development in relevant populations	