Beyond RV144 Efficacy Results: An Update

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Abstract

The RV144 efficacy trial conducted in Thailand provided the first evidence that an HIV vaccine could provide a modest level of protection against HIV acquisition (31.2% at 42 months of follow-up) in populations at low risk for HIV infection. Vaccine efficacy appeared to be higher (60%) at 12 months post vaccination, suggesting an early, but nondurable, vaccine effect. This breakthrough finding led to identification of immune correlates of risk, antibodies directed against the V2 loop, paving the way to new vaccine designs and clinical trials to better characterization of the vaccine-induced adaptive and innate humoral and cell-mediated immune responses in peripheral and mucosal compartments. Whether the RV144 correlates of risk are universal and apply to other populations at higher risk for HIV acquisition and other modes of transmission (rectal, injecting drug users) is unknown and remains to be explored. Future efficacy trials using a similar vaccine concept tested in high-risk heterosexual populations and in men having sex with men are planned.

1. RV144 Efficacy Trial

While new prevention strategies to control the epidemic and prevent new infections, including pre-exposure prophylaxis [1], antiviral treatment for prevention [2], and topical microbicides [3], the development of a preventive vaccine against HIV-1 remains among the best hope for controlling the HIV/AIDS pandemic [4].
1.1. Design

The Thai “Phase III” trial, RV144, provided the first evidence that an HIV vaccine could provide protective efficacy against HIV acquisition [5]. The prime-boost vaccine regimen consisted of a non-replicating recombinant canarypox vector, ALVAC-HIV vCP1521 prime, expressing gag, protease subtype B (LAI) and env gp120 CRF01_AE with a gp41 subtype B (LAI) transmembrane anchor, administered at 0, 1, 3, and 6 months and a bivalent AIDSVAX gp120 B/E (MN and A244 CRF01_AE) boost given at months 3 and 6. The gp120 Env variants used in AIDSVAX B/E were engineered with a deletion of the first 11 N-terminal amino acids (aa) with the addition of an HSV-2 leader and a 27-aa HSV-2 gD protein tag. This was a community-based trial initiated in 2003 in Rayong and Chon Buri provinces of Thailand. The vaccine regimen was safe and generally well tolerated [6]. The modified intent-to-treat analysis (excluding those randomized, but HIV-infected at the first vaccination visit) was conducted on 16,395 volunteers.

1.2. Efficacy

The analysis showed 31.2% efficacy after 42 months of follow-up. There was no effect on early post-infection HIV-1 RNA viral load or CD4+ T-cell count. A post-hoc analysis with Bayesian assumptions and statistics gave a 71% probability that RV144 showed efficacy [7]. In another post-hoc analysis, not included in the pre-specified analysis plan, vaccine efficacy appeared to be higher (60%) at 12 months post vaccination, suggesting an early, but nondurable, vaccine effect. The authors pointed out that future HIV vaccine trials should recognize potential interactions between challenge intensity and risk heterogeneity in both population and treatment effects [8]. One can speculate that the RV144 regimen was able to protect because the number of sexual contacts were limited in time and could be countered by a marginally efficacious vaccine, which might or might not hold true with communities at higher risk of sexual transmission and increased number of exposures such as MSM and female sex workers. A further analysis of the effect of vaccination on disease progression after infection showed a weak evidence of lower viral load and higher CD4+ count in the vaccine group. Interestingly, lower mucosal viral load was observed among vaccine recipients, primarily in semen. Vaccination did not affect the clinical course of HIV disease after infection [9].

1.3. Immunogenicity

Immune responses were assessed in a subset of volunteers (112 vaccine and 20 placebo recipients) 2 weeks after the fourth dose administration. Rates of positivity in the gp120 and p24 binding-antibody assays and the lymphoproliferation assay were similar to those in the phase II study. Binding antibody against Env was nearly uniformly present to MN and A244 strains, whereas p24 responses were less frequent. The stimulation index was significantly higher in vaccine recipients compared to baseline and placebo recipients. IFN-γ ELISPOT positive responses were measured in 25 (41%) vaccinees and were predominantly CD4+ T cell-mediated. Vaccination induced an HIV-specific response, as measured by IFN-γ ELISPOT assay to either Env or Gag antigen, in 19.7% of volunteers 6 months after the final dose of vaccine was administered. Responses were targeted within the HIV Env region, with 60% of vaccinees recognizing peptides derived from the V2 region of HIV-1 Env, which includes the α4β7 integrin binding site. Intracellular cytokine staining confirmed that Env responses predominated (63% of vaccine recipients) and were mediated by polyfunctional effector memory CD4+ T cells. Response rates for CD4+ Env-specific ICS were higher in the vaccine group than in the placebo group (32% vs. 2%). Proliferation assays revealed that HIV antigen-specific T cells were CD4+, with the majority (80%) expressing CD107a. HIV-specific T-cell lines obtained from vaccine recipients confirmed V2 specificity, polyfunctionality, and functional cytolytic capacity [10].
Neutralization was assessed with tier 1 and tier 2 strains of virus in TZM-bl and A3R5 cell assays. Neutralization of several tier 1 viruses was detected in both RV144 and Vax003, the latter being an efficacy trial conducted in injecting drug users in Bangkok, Thailand [11]. Peak titers were higher in Vax003 (2 weeks after the fourth inoculation of AIDSVAX B/E) than in RV144 and waned rapidly in both trials. ALVAC-HIV (vCP1521) priming followed by two boosts with gp120 protein was superior to two gp120 protein administrations alone, confirming a priming effect for ALVAC-HIV. Sporadic weak neutralization of tier 2 viruses was detected only in Vax003 using the A3R5 cell assay [12]. Non-response to vaccine was associated with DRB1*11 and DRB1*16:02 alleles. Vaccine recipients with HLA-DQ heterodimers encoded by DQA1*05:01 and DQB1*03:01 alleles, were less likely to produce neutralizing antibodies (NAb). These data suggest that the lack of response to a vaccine designed to induce clade-specific HIV NAb is associated with the presence of certain HLA class II alleles and heterodimers in some Southeast Asians [13]. These results suggest that the modest efficacy observed in RV144 might be mediated by other protective mechanisms either alone or in combination with weak neutralizing antibodies effective in low-risk heterosexual populations.

2. RV144 correlates of risk

2.1. Methodology

The efficacy observed in the RV144 trial provided the first opportunity to study immune correlations with vaccine efficacy against HIV. A large and diverse consortium of investigators systematically evaluated assays that detect antibody, innate, and cellular immune responses [14]. Initial pilot studies evaluated 32 assays of 17 types on the basis of reproducibility, non-redundancy, low false positive rate, and large dynamic range using 50-100 samples from uninfected RV144 volunteers collected at baseline and peak immunogenicity (80% of whom were vaccine and 20% placebo recipients). In order to optimize statistical power to show a correlation of risk between vaccinated persons who acquired versus those that did not acquire HIV-1 infection, 6 primary variables were identified for a case-control analysis using peak immunogenicity samples from vaccine recipients who acquired HIV-1 infection and controls that did not acquire infection.

2.2. Findings

Two of these variables correlated significantly with HIV-1 infection risk: plasma IgG binding antibody to scaffolded gp70 V1V2 envelope proteins correlated inversely with risk, while Env plasma monomeric IgA binding score correlated directly with risk, raising the hypothesis that IgA responses against Env and IgG responses directed against V1V2 may be mechanistically associated with RV144 vaccine regimen-mediated protection.

Binding antibody responses against the V2 region were further characterized. Using peptide microarray, surface plasmon resonance, and ELISA, 97% of 32 studied plasma samples from RV144 vaccine recipients 2 weeks post last vaccination contained antibodies that recognize V2 region synthetic peptides. Percent responders fell to 19% at 28 weeks post last vaccination, and V2 responses were significantly more frequent in vaccine recipients as compared to individuals naturally infected with HIV-1 [15]. In addition, Zolla-Pazner et al. described results from the 13 primary and exploratory assays associated with the correlates analysis [16]. All V2-related odds ratios were ≤1. Based on epitope mapping, V2 antibody responses are directed against a region including residues 165-178, which lies in the ‘crown’ of V2, and lies immediately N-terminal to the LDI tripeptide α4β7 binding motif at residues 179-181.
Neither low levels of V1V2 antibodies nor high levels of Env-specific IgA antibodies were associated with higher rates of infection than were found in the placebo group, suggesting there was no evidence for enhancement of infection risk in the overall study or associated with any of the case-control assay variables. In vaccinees with low levels of Env-specific IgA antibodies, four of the other five primary variables, IgG avidity, ADCC, neutralizing antibodies, and Env-specific CD4+ T cells, were inversely correlated with infection. The reasons for negative association between high levels of plasma IgA and protection are unclear. It has been hypothesized that IgA may block the action of IgG [17], in particular ADCC [18] and phagocytosis [19]. Vaccinees with IgA antibodies to the first conserved region C1 of gp120 had a higher risk of infection than vaccinees without these antibodies. The gp120 C1 region contains an epitope that can be a target on the surface of virus-infected cells for antibodies that mediate ADCC.

In an earlier Phase I trial, the RV144 regimen was shown to induce potent ADCC activity [20]. The combination of low plasma anti-HIV-1 Env IgA antibodies and high levels of ADCC inversely correlate with infection risk. One hypothesis is that the observed protection in RV144 may be partially due to ADCC-mediating antibodies. The majority of a representative group of vaccinees displayed plasma ADCC activity, usually blocked by competition with the C1 region-specific A32 Fab fragment. Using memory B-cell cultures and antigen-specific B-cell sorting, 23 ADCC-mediating non-clonally related antibodies were isolated from 6 vaccine recipients. These antibodies targeted A32-blockable conformational epitopes, a non A32-blockable conformational epitope, and the gp120 Env variable loops. Fourteen antibodies mediated cross-clade target cell killing. ADCC-mediating antibodies displayed modest levels of V-heavy (VH) chain somatic mutation (0.5-1.5%) and also displayed a disproportionate usage of VH1 family genes (74%), a phenomenon recently described for CD4-binding site broadly neutralizing antibodies [bNAb]. Maximal ADCC activity of VH1 antibodies correlated with mutation frequency. The polyclonality and low mutation frequency of these VH1 antibodies reveal fundamental differences in the regulation and maturation of these ADCC-mediating responses compared to VH1 bNAb [21]. The HIV-1 Env gp120 monoclonal antibody (MAb) A32 binds to the surface of transmitted/founder HIV-1-infected CD4+ T cells earlier in the course of in vitro infection than the gp120 Env MAbs 17b and 2G12. MAb A32 was able to mediate ADCC activity that was 4- to 6-fold higher than that of the other two anti-gp120 MAbs when either gp120-coated or HIV-1-infected target CD4+ cells were used. Antibodies that are blocked by A32 Fab comprise a majority of CD4-inducible ADCC-mediating antibody responses elicited during the course of HIV-1 infection [22].

2.3. Sieve analysis

Approximately 90% of incident infections in RV144 were CRF01_AE infections, also known as subtype E, which is the predominant circulating strain in Thailand and much of South East Asia [23]. A sieve analysis was performed on 1,025 genome equivalents from 121 RV144 participants who became HIV-infected during the trial (viruses were not transmitter/founder since the mean time to last negative visit was approximately 3 months.) Viral escape was associated with V2 and C1 region sequence changes. Sieve effect was associated with aa 69-95 in the C1 region. Targeted V1/V2-focused analysis identified two signatures of vaccine pressure within the V2 loop corresponding to sites 169 and 181. Intriguingly, VE against viruses matching the vaccine at position 169 was 48% whereas VE against viruses mismatching the vaccine at position 181 was 78%. Although the full interpretation of these results remains unclear, the sieve analysis provides additional evidence supporting the hypothesis that vaccination-induced immune responses directed against the V2 loop were associated with protection [24].
3. Implications for future clinical trials and the way forward

3.1. Unanswered questions

Several questions remain unanswered and should deserve further analysis in RV144 and future clinical trials and consideration for the design of improved Env subunit vaccines.

The RV44 analysis of the correlates of risk is based on immune measurements performed on blood specimens. Additional analysis of the V1V2 responses using different scaffolds and V2 sequences from various subtypes as well as the possible role V2 antibodies in blocking the attachment of the virus to the α4β7 motif may provide tremendous information on cross-clade V2 binding antibodies and predictive value as correlate of risk in a subsequent statistical analysis. What is the role of IgA, and is differential induction of IgA a feature specific for the immunogen, the route of delivery, or the adjuvant? What immune responses are vaccine-elicited in the mucosal entry points (vaginal, rectal) of the virus for sexual transmission, in particular IgG and dimeric IgA to V1V2 binding antibodies remain crucial to elucidate. Does the HIV-specific antibody response in mucosal secretions hinder HIV mobility? Can vaccine-induced immune responses be magnified and sustained by additional Env subunit boosts and more potent adjuvants? Can human results be recapitulated in non-human primate studies? Testing V2 monoclonal antibodies in SHIV challenge studies may offer additional clues to the RV144 findings. Can V2 antibodies be elicited by Env subunits from different HIV subtypes and circulating recombinant forms?

3.2. Improved envelope subunit vaccines

The RV144 protein immunogens (A244-rp120, MN-rgp120) were modified by an N-terminal 11 amino-acid deletion (Δ11) and addition of a HSV (Herpes simplex virus)-gD protein derived tag (gD). Analysis of A244 gp120, with or without Δ11 or gD, demonstrated that the Δ11 deletion, without the addition of gD, was sufficient for enhanced antigenicity to gp120 C1 region, conformational V2 and V1/V2 gp120 conformational epitopes. RV144-vaccinee sera IgG bound more avidly to A244 gp120 Δ11 than to the unmodified gp120 and their binding was blocked by C1, V2 and V1/V2 antibodies. Rhesus macaques immunized with the three different forms of A244 gp120 proteins gave similar levels of gp120 antibody titers, although higher antibody titers developed earlier in A244 Δ11 gp120 immunized animals. Conformational V1/V2 mAbs gave significantly higher levels of blocking of plasma IgG from A244 Δ11 gp120 immunized animals than IgG from animals immunized with unmodified A244 gp120, thus indicating a qualitative difference in the V1/V2 antibodies induced by A244 gp120 [25].

Avidity of Env antibodies may also play a protective role against HIV acquisition in humans. Macaques vaccinated with an immunization regimen intended to mimic the RV144 trial were challenged intrarectally to a dose of SIVmac251 that transmits few virus variants, similar to HIV transmission to humans. Three of the eleven macaques vaccinated were protected from SIVmac251 acquisition. Sera from protected animals had higher avidity antibodies to gp120, recognized the variable envelope regions V1/V2, and reduced SIVmac251 infectivity in cells that express high levels of α4β7 integrins, suggesting a functional role of antibodies to V2 [26].

3.3. Future clinical studies

Several clinical trials have been designed to address these questions in particular assessing immune responses in the mucosal compartments. RV305 is testing the effect of long-term boosts (AIDSVAX B/E
vs. ALVAC-HIV vs. ALVAC-HIV/AIDSVAX B/E combination) in RV144 vaccine recipients. RV306 will recapitulate the RV144 regimen with different additional 12-month boosts (as in RV305). RV328 will test AIDSVAX B/E in a Vax003 regimen.

In 2007, HIV prevalence among MSM in Bangkok and Chiang Mai was 30.7% and 16.9%, respectively, essentially unchanged from 2005 [27]. The HIV prevalence found in subsequent studies ranged from 5.5%-28.3% with an incidence rate of 8.2 per 100 person years [28 29], and 6% in Bangkok between 2006-2008 [30]. Among MSM and transgender sex workers from Pattaya screened for a cohort study, HIV incidence is 6.2 and 7.6 per 100 person years, respectively (Merlin Robb, personal communication). New cohort studies are now planned to prepare the conduct of future efficacy prevention trials.

4. Conclusion

Considerable efforts remain ahead that will require major and sustained funding and political willingness in order to bring an HIV vaccine to licensure. The manufacturing of improved Env subunits formulated with more potent adjuvants remains one of the critical issues.

Whether the RV144 correlates of risk are universal and apply at least partially to other populations at higher risk for HIV acquisition and other modes of transmission (rectal, injecting drug users) is unknown and remains to be explored. Future efficacy trials using a similar vaccine concept tested in high-risk heterosexual populations (southern Africa) and in men having sex with men (Thailand) may answer this question.

Disclaimer

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References


