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Overview of STEP and Phambili trial results: two phase IIb test of concept studies investigating the efficacy of MRK ad5 gag/pol/nef sub-type B HIV vaccine

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Abstract

Purpose of review—Two phase IIb test of concept studies evaluated the replication-defective adenovirus type 5 vaccine MRK gag/pol/nef HIV vaccine to prevent infection or decrease early plasma viral load in disparate populations. The Step study enrolled men and women in the Americas, Caribbean and Australia; the Phambili trial enrolled men and women in South Africa, where the modes of sexual transmission and HIV-1 risk, sub-types of HIV-1, and background Ad5 seroprevalence differed.

Recent findings—Vaccination in both studies were stopped, after the first interim efficacy analysis of the Step study crossed pre-determined non-efficacy boundaries. Neither trial demonstrated a decrease in HIV acquisition nor decreased early plasma viral load in vaccinees compared to placebo recipients. Post-hoc analyses of men enrolled in the Step study showed a larger number of HIV infections in the sub-group of vaccinated men who were Ad5 seropositive and uncircumcised compared to a comparable placebo group. This was not demonstrated in the Phambili study, where most men were heterosexual, while most in Step were homosexual/bisexual. Further analysis of the Step study has yet to explain the effect of Ad5 seroprevalence on increased HIV-1 susceptibility in men receiving the vaccine. However, promising vaccine effects on early viral control were seen, and the possibility of effects on early viral load setpoint in women in Phambili was seen.

Summary—These trials have provided a number of lessons about the importance of clinical trials in the HIV vaccine discovery process, and insight into the type and level of immune response that will be required for control of viral replication.

Keywords

Phase IIb test of concept trials; HIV vaccines; ad5 vaccines

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Conflict of interest statement GG and AD declare that they have no conflict of interest.

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Introduction

An effective HIV vaccine could prevent HIV infection and/or reduce viral load and clinical disease progression post-infection, and to achieve this, may need to stimulate both protective antibody and T cell responses. To date, adenovirus type-5 is the best-studied serotype of adenoviral vaccine vectors, and replication-defective Ad 5 viruses are being studied for HIV vaccine vectors based on their ability to stimulate anti-HIV T-cell responses [1]. Given the importance of cell mediated immune (CMI) responses in the control of viral replication and disease progression in long term non-progressors and in non-human primate challenge models, the MRK Ad5 sub-type B HIV-1 gag/pol/nef vaccine was designed to elicit CMI responses. In phase I clinical studies, the Ad5 vector-based vaccines were found to be among the most immunogenic of CMI vaccines [2,3].

Based on promising preclinical studies in non-human primates and demonstrated safety and strong cellular immunity in early clinical trials, two phase IIb test of concept trials were designed. These assessed the efficacy of the MRK Ad5 HIV-1 gag/pol/nef vaccine in areas of the world where different strains of HIV-1 predominate, where the seroprevalence and average neutralizing titer to Ad5 differed, and where the mode of sexual transmission varied. The first study, known as the Step study or Merck 023/HVTN 502, was conducted in regions of the world where clade B is the predominant HIV-1 sub-type (North America, the Caribbean, South America and Australia), and included homosexual men who had multiple partners or who practiced unprotected anal intercourse and high-risk heterosexual men from the Caribbean and women [4] who practiced unprotected sexual intercourse with high risk partners. The second study, called the Phambili or HVTN 503 study, was conducted in South Africa, where the circulating HIV-1 sub-type is clade C, and where the predominant mode of transmission is via unprotected heterosexual sex.

Vaccination and enrolment into these studies were terminated in September 2007 at the first interim review of the Step study by the independent Data and Safety Monitoring Board for non-efficacy in achieving its primary endpoints. In addition, in further post-hoc analyses there appeared to be a trend of increased HIV-1 infections in Ad5 seropositive men and uncircumcised men who had received the vaccine. Based on these data, the studies were unblinded, and participants were informed of their treatment allocation. In this article, we explore further the factors associated with HIV-1 infection among Step and Phambili study participants, as well as assessing the impact of vaccination on the natural history of HIV-1 infection in both the Step and Phambili studies. We also assess risk reduction behavior over time, the effect of discontinuation of enrolment/vaccination and the uptake of circumcision as a HIV prevention method in Phambili.

Pre-existing immunity to Ad5 and HIV-1 infection

In certain sub-groups of Step study participants, notably those with neutralizing antibodies to Ad5 at baseline and those that were uncircumcised, HIV acquisition appeared to be higher among vaccinees as compared to placebo recipients (The unadjusted HR for risk of HIV-1 acquisition in participants receiving vaccine compared with those receiving placebo was highest in men who were uncircumcised and Ad5 seropositive (n=620, HR 3.9 [95% CI 1.3–11.9]). Risk was intermediate in men who were uncircumcised and Ad5 seronegative (n=168, HR 3.3 [0.7–15.8]) and in men who were circumcised and Ad5 seropositive (n=421, HR 1.6 [0.7–3.8]). Vaccinees who were both circumcised and Ad5 seronegative did not appear to be at increased risk (n=578, HR 0.7[0.3–1.4]) [4]. This initial finding of potential enhancement of HIV-1 acquisition in vaccine recipients with pre-existing immunity to Ad5 was unexplained, and not predicted by prior clinical studies [5]. It is possible that this

association occurred by chance; the p value for interaction (i.e., that the vaccine effects were different among the Ad5 seropositive and Ad5 seronegative male participants) was 0.08.

To explore the potential effect of Ad5 immunity on vaccine effects, a number of analyses were undertaken. First, given a trend toward higher baseline Ad5 titers and lower HIV incidence in the placebo group, the relationship of baseline Ad5 titers and HIV incidence was studied in unvaccinated populations. A multivariate analysis of placebo recipients in the Step study found no association of pre-existing Ad5 serostatus with HIV acquisition risk, after controlling for baseline demographic and risk variables [6]. This lack of a relationship between baseline Ad5 neutralizing antibody titer and HIV acquisition risk was confirmed in a nested case-control study in 2 longitudinal cohorts of MSM: the Multi-center AIDS Cohort Study (MACS) and HPTN039 [Curlin M et al, unpublished data]. The relative risk of incident HIV infection among Ad5 seropositives vs. Ad5 seronegatives were 1.1 ($p = 0.57$) and 1.0 ($p = 0.99$) in the MACS study and HPTN 039, respectively; furthermore, no association was seen between HIV acquisition and baseline neutralizing Ad5 antibody titer, when treated as a continuous variable [6].

An initial hypothesis of the increased risk of HIV acquisition among Ad5 seropositive vaccinees compared to placebo recipients was that participants who were Ad5 seropositive would have higher levels of Ad5-specific CD4+T lymphocyte response, which may have expanded rapidly following vaccination. This expanded population of activated lymphocytes could potentially serve as targets for HIV infection, especially if they homed to mucosal sites. Benlahrech and colleagues explored this hypothesis in an in vitro study using blood from 20 healthy volunteers [7]. Ad5 and Ad11 antibody titers were measured and dendritic cells were pulsed in vitro with replication defective Ad5 or Ad11, then co-cultured with autologous lymphocytes. Results from this study showed that although adenovirus-specific cytokine responses did not correlate with Ad5 titer, there was a correlation between CD4+T-cell proliferation, stimulated by Ad5 or Ad11, and Ad5 neutralizing antibodies (titers above 200). In addition, this study showed that Ad5 or Ad11 re-stimulated memory CD4 cells were more susceptible to HIV-1 infection than unstimulated cells in vitro. While these in vitro manipulations are of interest, the relevance of these findings to the Step study are not known.

For example, data from two in vivo studies among participants who received the same vaccine used in the Step study, however, found that Ad5 specific CD4+T cells were unlikely to play a role in the possible increased susceptibility to HIV-1 infection seen in the Step study [8,9]. The presence of pre-existing Ad5 neutralizing antibodies was found not to predict presence of Ad5-specific CD4+ T-cells – either before or after vaccination. Ad5 specific CD4+T-cells within Ad5 seronegative and seropositive subjects were found to expand in response to Ad5 vector administration, and Ad5 seronegative subjects uniformly became Ad5 seropositive after the first vaccination (yet no enhanced susceptibility was noted in this group in the Step study) [8]. These findings are restricted to circulating CD4+Tcells, and do not address any potential differences in activated Ad5-specific CD4+T cells within mucosal tissues after vaccination. Results from a second clinical study using the Merck Ad5 trivalent vaccine found no evidence of CD4+ T cell activation after vaccination. This study showed that Ad5 specific neutralizing antibodies prior to vaccination were not correlated with Ad5-specific cellular immune responses, and that Ad5 specific T lymphocyte responses following vaccination were lower in Ad5 seropositive as compared to Ad5 seronegative subjects [9].

Alternatively, Ad5 specific neutralizing antibodies could have influenced the risk of HIV infection through immune complex formation following vaccination, and subsequent alterations in inflammatory responses [10]. In vitro studies found that immune complexes

formed from adenovirus serotype 5 incubated with Ad5 neutralizing antibodies were capable of inducing dendritic cell maturation and were associated with increased HIV infection in dendritic cell – T cell cocultures.

Another hypothesis to explain potential interactions between the Ad5-based vaccine and lack of circumcision speculates that Ad5 specific neutralizing antibodies could have influenced the risk of HIV infection through immune complex formation following vaccination, and subsequent alterations on dendritic cell maturation and inflammatory responses. Additional data from the Step study collected through January 23, 2009, show that the overall risk of HIV acquisition associated with vaccination has decreased over time through January 2009 [6]. Additional analyses are underway evaluating data through the end of follow-up (December 2009), to evaluate whether the trend toward increased risk in the Ad5 seropositive vaccinees was likely due to a spurious association that has diminished with more endpoints, or whether this reflects a true increase in risk peri-vaccination that has diminished with increased follow-up. Data from the Phambili study found no increased risk of HIV acquisition among vaccinees with higher baseline Ad5 titers, whether categorized using either 18 or 200 as a cut-point and adjusting for Ad5 had little effect on the treatment hazard ratio [11]. Much has been learned about the potential role of pre-existing immunity to viral vectors in these studies, which may be important in moving forward with vaccine candidates for HIV and other disease. These issues can be difficult to study in non-human primate models, particularly when the host range of the viral vector does not extend to these animal models. Earlier high-dose non-human primate challenge models were also unable to assess adequately any risk for increased acquisition associated with the vaccine; low dose mucosal challenge models can be structured to evaluate both protection and enhanced infection associated with vaccination.

Circumcision and HIV-1 acquisition

Surprisingly, in the interim analysis of the Step study, uncircumcised men who received the vaccine appeared to be at greater risk for HIV acquisition than uncircumcised placebo recipients (Relative hazard 3.8 [95%CI 1.5–9.3]) [4]. The increased risk of HIV acquisition appeared to be especially concentrated among men who reported unprotected insertive anal sex with HIV positive or unknown serostatus partners, lending indirect support to the possibility that uncircumcised vaccinated men were at increased risk for acquiring HIV through unprotected insertive anal sex. However, in the Phambili study, in which approximately 30% of men were circumcised at baseline, and an additional 26% were circumcised during the study [11,12], circumcision status did not predict infection nor modify the effect of treatment [Gray G, personal communication]. Most of the men in the Phambili study were heterosexual, with very few reporting unprotected anal sex practices; if the risk of acquisition were increased for men who were having unprotected insertive anal sex, then it might have also been seen in the Phambili population. In Step, the increased hazard among vaccinees compared to placebo recipients also appears to be decreasing in the subgroup of uncircumcised men in follow-up through January 2009 [6]. Again, further analyses using the complete dataset through December 2009 are currently underway; efforts are being taken to differentiate between a spurious result that has diminished with a larger number of endpoints, and an early effect of the vaccine that has waned with increased duration from the time of the vaccination series. This study has highlighted the importance of studying mucosal immune responses to vaccination, including responses in the foreskin.

Vaccine effects on viral load set-point

Initial analyses based on 88 individuals from the Step study who were HIV-infected at the time of the DSMB review (9/2007) indicated no vaccine effect on early HIV viral load

(mean of VL at 8 and 12 weeks post-diagnosis of HIV infection). Similarly, no difference in early HIV viral load was seen among HIV-infected vaccine- or placebo recipients detected in the Step study through January 23, 2009 (data available for 126 HIV-infected men). Among men with Ad5 titers ≤ 18 mean log viral load in vaccine and placebo recipients was 4.58 and 4.37, respectively; the corresponding values among men with Ad5 titers >18 were 4.46 and 4.70, respectively.

In the Phambili study, the geometric mean of the viral load set-points was 21,110 copies/mL for the vaccine arm (N=30) and 33,304 copies/mL for the placebo arm (N=24) ($p=0.39$) (Figure 1). Women tended to have lower viral load set-points than men, but the difference was not statistically significant ($p = 0.18$). Although also not statistically significant, viral load setpoints were lower in women who received the vaccine as compared to placebo recipients ($p=0.18$) [11]

Disease Progression

Among the first 87 male study participants who acquired HIV infection during the Step study, there was no difference in HIV disease progression between vaccine and placebo recipients during two years of follow up (through September 22, 2009). Time to initiation of antiretroviral therapy, HIV RNA levels, CD4+ T-cell counts, and AIDS-free survival were similar in vaccine and placebo recipients. There was no evidence that the vaccine effect on CD4 counts or viral load was modified by Ad5 sero-status or circumcision status [Fitzgerald, unpublished data,].

In the Phambili study, using a CD4 cut-off of 350, a significant difference between treatment arms was observed at week 2 and 3 months post-diagnosis. At 6 months, the percentage of participants having CD4 below 350 is still lowest for the vaccine arm, but the difference is no longer statistically significant (Table 1) [11].

Risk reduction behavior, circumcision access and effects of unblinding on risk behavior and trial participation

Self-reported risk behavior in the Step study prior to unblinding did not differ by treatment assignment; thus any difference in incidence among vaccinees and placebo recipients does not appear attributable to differences in risk behavior.

Data from the Phambili study demonstrates that sexual disinhibition did not occur prior to unblinding [13]. In both placebo and vaccine arms, significant reductions in number of sexual partners ($p<0.001$) and unprotected sex ($p<0.001$) were observed before vaccination was halted. Post-unblinding, men were more likely to report behavior change; reported changes included increased condom use, encouraging partner testing, and reduced number of sexual partners. More vaccinees as compared to placebo recipients agreed/strongly agreed that they were more likely to get HIV than most people ($p<0.001$), and this was attributed to having received the vaccine. Post-unblinding, participants believed that site-participant communication was appropriate, and felt they had made the right decision in enrolling [14]. The uptake of male circumcision as a prevention method varied by site and inversely with baseline circumcision prevalence [15]. One in four uncircumcised men in the study opted to become circumcised post-enrolment, and this did not differ by treatment allocation, or the period of time before or after treatment unblinding.

Conclusion

The Step and Phambili studies moved the HIV vaccine field forward in important ways. Despite a more robust CMI response from this vaccine than other vaccines in clinical trials, and data from non-human primate models suggesting that such an immune response might lead to control of viral replication, this vaccine failed to provide a lasting reduction in HIV viral load in MSM and heterosexual men and women. However, the studies have provided clues about potential early viral effects, with evidence of impact on the founding virus among breakthrough infections, and potential transient reduction in early viral load measures among women.

Several new questions were raised from these studies, including the potential for increased acquisition and potential mechanisms, the importance of understanding the impact of immune responses to viral vectors, and the potential role of mucosal immune responses (including in foreskin) in vaccine effects. None of these questions had been studied in non-human primate studies, but they do point to several important roles for such studies, including a focus on early events post-infection, and sampling multiple mucosal specimens to evaluate potential immune correlates. Phase IIb trials have an important role in vaccine discovery, and may provide insight into the type and level of immune response that will be required for protection against HIV acquisition and control of viral replication. In addition, the trials serve as a benchmark for timely review of trial data and active participation of the greater vaccine research community in analysis of study data and specimens.

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Figure 1. Viral load Setpoint by Treatment Arm in the Phambili study

The HIV viral load setpoint was defined as the geometric mean of viral load at assessed at ~2 months through ~3 months after detection of infection for individuals not taking antiretroviral therapy. Viral load in vaccinees did not differ from that among placebo recipients.

Hollow symbols indicate that viral load set-point is estimated as follows:

- Either month 2 or month 3 viral load is used
- If month 2 or month 3 are missing, 1 month post diagnosis is used
- If month 1-3 are missing, the average of the diagnostic samples are used

Table 1

Effect of vaccination status on progression to CD4 counts of 350 or less in the HVTN 503/Phambili study

Treatment Group	Number and % participants with CD4 counts of 350 or less after HIV diagnosis		
	2 weeks post HIV+ diagnosis N=42	3 months post HIV+ diagnosis N=46	6 months post HIV+ diagnosis N=41
Vaccine	2/24 (8.3%)	3/26 (11.5%)	6/24 (25%)
Placebo	7/18 (38.9%)	10/20 (50%)	9/17 (52.9%)
P value	0.02	0.007	0.10