**BACKGROUND**

Heterologous prime boost vaccine regimens offer a promising approach to improving T cell and antibody responses. Homologous and heterologous prime boost regimens were compared using two Adenovirus vectors expressing two HIV-1 subtypes A-Env.

**METHODS**

**Vaccine candidates.** The Ad26.EnvA vaccine was manufactured by Crucell Holland BV (The Netherlands) and was a replication-deficient adenovirus type 26 (Ad26) constructed to contain an HIV-1 Clade A (92RW020) Env gene encoding a modified envelope gp140 protein.

The Ad35-Env vaccine was manufactured by Transgene (France) and was a recombinant replication-competent adenovirus type 35 (Ad35) constructed to contain an HIV-1 subtype A (TZA173) Env gene encoding a modified gp140 protein.

Both vaccines were previously assessed in phase I human clinical trials (ref 3).

The amino acid sequence homology was 72% between the 2 Env.

**Trial Design.** 217 subjects were enrolled in this trial of whom 173 received an active study vaccine (44 received placebo) as shown in Table 1. Ad26.EnvA and/or Ad26-Env were administered at 5 × 10^10 viral particles intramuscularly. The study was conducted in three geographic regions (US: East and South Africa) and tested homologous and heterologous vaccine regimens at two different schedules. US volunteers (Groups A-D) received homologous A26.EnvA/Ad35-Env or Ad35-Env/Ad26-Env regimens at 0/3- or 0/6-month intervals. African volunteers (Groups E-L) received the same heterologous or homologous regimens consisting of two Ad26-Env or two Ad26-Env at 0/3 months (Table 1 for modular trial schema).

**Immunogenicity.** The safety and immunogenicity of Ad26.EnvA and Ad35-Env at 5 × 10^10 vp each, in homologous and heterologous regimens and at two dose schedules (0, 3 and 0, 6 months) was assessed (see table 2 for modular trial schema). For information on the safety of the vaccine regimens see paper By Elsennie Karita et al. Serum and peripheral blood mononuclear cells (PBMC) were processed at each clinical site. Cryopreserved PBMC and frozen serum samples were shipped to three central laboratories where immunogenicity assays were performed: the IAI Human Immunology Laboratory (HIL) at Imperial College, London, Beth Israel Deaconess Medical Center (BIDMC), Boston and the HIV Vaccine Trials Network Laboratory at Fred Hutchinson Cancer Research Center (FHRC), Seattle.

**ELISPOT.** Intracellular cytokine secretion assays, Ad26 and Ad35 neutralization assays and Env ELISA were performed according to each laboratory’s standard operating procedures (SOP), analytical plans and analysis criteria under GCLP accredited or other quality control strategies by blinded operators (see Figure 2). Immunology methods are described in references 1:3

**RESULTS**

**Figure 2. Antibody ELISA.** Two proteins were used for the ELISA assays: EnvA 92RW020 (Figure 2a) exactly matched to the Ad26.EnvA (VRC, Bethesda, MD) and UG37 Env (Figure 2b) (Polymun Scientific, Vienna, Austria). A titer of 1/100 was defined as positive for either of the proteins. X-axis: B = baseline, 1 = 4 weeks post 1st, 2 = 2 weeks post 2nd

• Overall Post 1st vaccine response: 33-100%

• Overall Post 2nd vaccine response: 97-100%

• Up to 2-fold boost of Env Antibodies from 1st to 2nd vaccine

• Heterologous and homologous regimens comparable

• Responses between the 3 and 6 month schedule were comparable

• Response rate for matched Env slightly better than non-matched Env

• No correlation with Ad35 NAB or Ad26 NAB and Env antibody responses

• The response rate at baseline was 0% for Env A UG37 and <1% for Env 92RW020

**Figure 3. ELISPOT assay.** Testing was conducted at WI01/FHRC Laboratory and was performed only on samples from groups E at 4 weeks post 1st and 2nd vaccine with peptide pools matched to the Ad26-Env and Ad35-Env, in addition PTE peptides were used. Results were available for each of the three peptide pools separately, data for Ad26-Env is shown in Figure 4. Overall for any Env, CD4+ ICS response rates ranged from 0% to 71% and CD8+ ICS response rates ranged from 0% to 80% with the highest responses post second vaccine. Balanced CD4 and CD8 responses were seen across regimens, but with differences across regimens observed depending on the peptide set used.

**Figure 4. ICS assay.** Testing was conducted at WI01/FHRC Laboratory and was performed only on samples from groups E at 4 weeks post 1st and 2nd vaccine with peptide pools matched to the Ad26-Env and Ad35-Env, in addition PTE peptides were used. Results were available for each of the three peptide pools separately, data for Ad26-Env is shown in Figure 4. Overall for any Env, CD4+ ICS response rates ranged from 0% to 71% and CD8+ ICS response rates ranged from 0% to 80% with the highest responses post second vaccine. Balanced CD4 and CD8 responses were seen across regimens, but with differences across regimens observed depending on the peptide set used.

**Summary**

Env antibody responses were identified in nearly all volunteers (in all 3 regions) with a log increase in titer after the 2nd immunization. T cell responses magnitudes were modest across all regions and regimens. Immune responses were comparable between the 3 and 6 month schedules. Heterologous and homologous regimen immunogenicity were comparable and not impacted by baseline vector immuno. But may have been impacted by potential lack of immunological cross-reactivity between the two Env.

**AFFILIATIONS AND ACKNOWLEDGMENTS**

1. *International AIDS Vaccine Initiative (IAVI), Human Immunology Laboratory, UK*, *Brigham and Women’s Hospital, Harvard Medical School, USA*, *Fred Hutchinson Cancer Research Center, USA,* *VRI, USA*, *Beth Israel Deaconess Medical Center, Harvard Medical School, USA*, *WHO Collaborating Centre for Reovirus & ZMBV Research Group, Zambia, Kenya AIDS Vaccine Initiative, University of Nairobi, Kenya*, *The Desmond Tutu HIV Centre Institute of Infectious Disease and Molecular Medicine Faculty of Health Sciences, South Africa*, *Perinatal HIV Research Unit, South Africa*, *The Aurum Institute for Health Research, South Africa*, *National Institute of Allergy and Infectious Diseases, USA*, *Crucell Holland BV, Netherlands*, *The EMIRIS Corporation, Rockville (MD), USA*, *Global BioSolutions, Zwolle, Netherlands*

Thanks to all the dedicated participants across the trial sites, the staff at the clinical sites and immunology support laboratories. Thanks to EMIRIS and SCHWPP for data analysis and clinical trial database expertise. Thanks to the United States Agency for International Development, to the National Institutes of Health and to the Ragon Institute of MGH for their support.

**REFERENCES**