Immunogenicity of homologous and heterologous regimens of Ad26-EnvA.01 and Ad35-EnvA HIV vaccines in HIV-uninfected volunteers in the US and Africa

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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 subtype A Envs.

METHODS

Vaccine candidates. The **Ad26.ENVA** vaccine was manufactured by Crucell Holland BV (The Netherlands) and was a replication-deficient adenoviral 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-incompetent adenoviral 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 (refs1-3).
The amino acid sequence homology was 72.7% between the 2 Envs.

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 Ad35-Env were administered at 5 x 10^{10} viral particles intramuscularly. The study was conducted in three geographic regions (US, East and South Africa) and tested heterologous and homologous vaccine regimens at two different schedules. US volunteers (Groups A-D) received heterologous (Ad26.EnvA+Ad35-Env or Ad35-Env+Ad26.EnvA) regimens at 0/3- or 0/6-month intervals. African volunteers (Groups E-L) received the same heterologous regimen or homologous regimens consisting of two Ad26-EnvA or two Ad35-Env at 0/3 months (see table 1 for modular trial schema).

Immunogenicity. The safety and immunogenicity of Ad26.ENVA and Ad35-ENV at 5 x 10^{10} vp each, in homologous and heterologous regimens and at two dose schedules (0, 3 months and 0, 6 months) was assessed (see table 1 for modular trial schema. For information on the safety of the vaccine regimens see poster **By Etienne 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 centralized laboratories where immunogenicity assays were performed; the IAVI 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. IFN-γ 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 schemes by blinded operators (see Figure 2). Immunology methods are described in references 1-3.

Table 1. Trial Design

	Group	Vaccine/ Placebo	Month 0	Month 3	Month 6
US	A	10/3	Ad26	-	Ad35
	B	10/3	Ad35	-	Ad26
	C	10/3	Ad26	Ad35	-
	D	10/3	Ad35	Ad26	-
East Africa	E	16/4	Ad26	Ad35	-
	F	16/4	Ad35	Ad26	-
	G	16/4	Ad26	Ad26	-
	H	16/4	Ad35	Ad35	-
South Africa	I	16/4	Ad26	Ad35	-
	J	16/4	Ad35	Ad26	-
	K	16/4	Ad26	Ad26	-
	L	16/4	Ad35	Ad35	-

Figure 1. Immunogenicity Assays

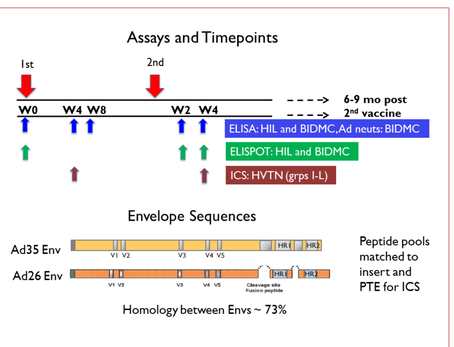


Figure 2a. EnvA 92RW020 ELISA

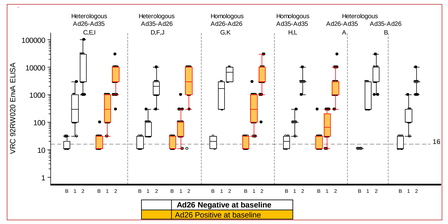


Figure 2b. EnvA UG37 ELISA

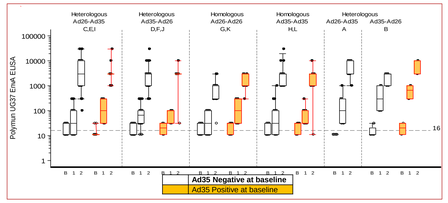
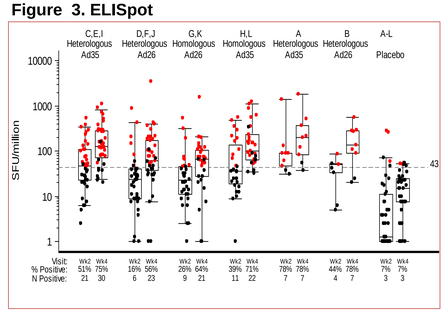


Figure 3. ELISpot



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 EnvA (Figure 2b) (Polymun Scientific, Vienna, Austria). A titer of ≥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 20-fold boost of Env Antibodies from 1st to 2nd vaccine
- Heterologous and homologous regimens comparable
- Responses between the 3 and 6 month schedules 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 <4% for EnvA 92RW020

Figure 3. T Cell Responses by IFN-γ ELISpot. Tested at 2 and 4 weeks post 2nd vaccine at the HIL and BIDMC laboratories respectively. Subtype A strain Env TZA173 and Env RW020 peptide pools matched to the Ad35-Env (2 pools) and Ad26.EnvA (one pool) were used and results reported for each of the three peptide pools separately. Data are shown for the Ad26.EnvA RW020 peptide pool (Figure 3).

- 4 weeks Post 2nd vaccine response rate: 44 -100%
- Heterologous and homologous regimens comparable
- Depends on Env peptide pool used ie matched vs non-matched (Ad26.EnvA vs Ad35-Env)
- Magnitude and response rate trend US > South Africa ~ East Africa
- No correlation with Ad35 or Ad26 NAbs and T cell responses

Figure 4. Multiparameter Flow Cytometry. Testing was conducted at HVTN/FHRC Laboratory and was performed only on samples from groups I-L at 4 weeks post 1st and 2nd vaccine with peptide pools matched to the Ad35-Env and Ad26-EnvA, in addition PTE peptides were used. Results were available for each of the three peptide pools separately, data for Ad26-EnvA is shown in Figure 4. Overall for any Env, CD4+ ICS response rates ranged from 0% to 71.4% 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

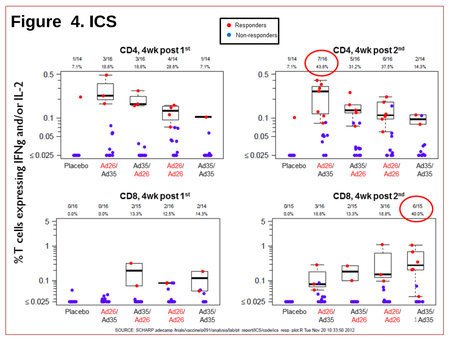
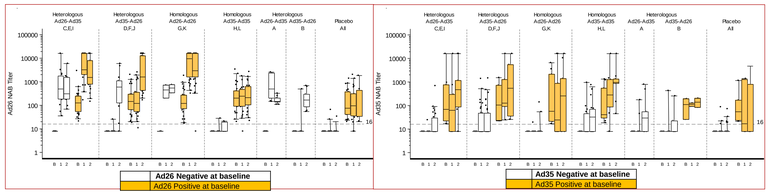


Figure 5. Neutralizing Antibody (NAb) Titers at 4 Weeks Post Each Vaccination



A titer of ≥16 was defined as positive. X-axis; B = baseline, 1 = 4 weeks post 1st, 2 = 4 weeks post 2nd

- Induction of Ad26 NAbs in the majority of vaccinees after two Ad26.ENVA immunizations
  - Ad26 NAb titers and response rates: East Africa ~ South Africa > US
- Induction of Ad35 NAbs in a subset of vaccinees after two Ad35-ENV immunizations
  - Ad35 NAb titers and response rates: South Africa > East Africa > US
- NAb titers and response rate Ad26 > Ad35 (baseline and vaccine-elicited)

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 response 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 immunity, but may have been impacted by potential lack of immunological cross-reactivity between the two Envs.

AFFILIATIONS AND ACKNOWLEDGMENTS

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