International AIDS Vaccine Initiative

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AIDS VACCINE2011 Bangkok, Thailand

Safety and immunogenicity of DNA and MVA HIV-1 subtype C vaccine prime-boost regimens: A Phase I trial in HIV-uninfected Indian volunteers

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ABSTRACT

With over 2.5 million HIV-infected people and evidence of ongoing transmission, the search for a preventive HIV vaccine for Indian people remains a high priority. We previously reported moderate immunogenicity of an MVA-based HIV vaccine in Indian volunteers. Other studies suggest that a superior immunogenicity can be induced by heterologous prime-boost regimens. We assessed the safety and immunogenicity of HIV-1 subtype C based DNA and MVA prime-boost regimens in Indian adults. Volunteers were randomly assigned to receive 2 doses of DNA followed by 2 doses of MVA [Group A] or 3 doses of MVA [Group B].

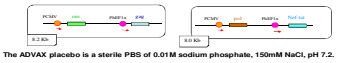
Safety: Local and systemic reactogenicity profiles were comparable between groups, and were mostly mild and transient. No serious adverse events were reported.

Immunogenicity In group A, vaccine recipients IFN-γ ELISPOT responses were detected in 0%, 25%, 100% and 100% participants post 1st, 2nd, 3rd and 4th vaccinations and in 60%, 63.6% and 91.7% of group B participants post 1st, 2nd and 3rd vaccinations, respectively. Responses were directed to multiple HIV proteins (mean magnitude of 108 – 250 SFU/10^6 PBMC) in most volunteers. HIV-specific antibodies were detected in 10/12 and 11/12 vaccine recipients in groups A and B, respectively, and neutralizing antibodies to tier-1 viruses were detected against HIV SF162 and MW-965 in most individuals.

MATERIAL & METHODS

Vaccine Constructs

ADVAX (China HIV-1 subtype C/B')



The ADVAX placebo is a sterile PBS of 0.01M sodium phosphate, 150mM NaCl, pH 7.2.

TBC-M4 (India HIV-1 subtype C)

The MVA placebo is a sterile 0.01M PBS (150mM NaCl) pH 7.0 containing 10% glycerol.

A phase I trial was conducted in 32 healthy HIV-uninfected adult volunteers of which 16 were enrolled at the National AIDS Research Institute [NARI], Pune, India and 16 at the National Institute for Research on Tuberculosis [Previously Tuberculosis Research Center], Chennai. Healthy HIV-uninfected volunteers (12 vaccine, 4 placebo per group) were randomly assigned to vaccine or placebo in group A (DNA at 0, 1 and MVA at 3, 6 months) or group B (MVA at 0, 1, 6 months). All vaccines were administered intramuscularly in the deltoid muscle. Reactogenicity was assessed at 3, 7 and 14 days post-vaccination; adverse events up to 9 months and serious adverse events through out the trial. T-cell and antibody responses were assessed pre and 1 and 2 weeks post each vaccination, then at 9, 12 and 18 months.

An IFN-γ ELISPOT assay was performed on fresh and cryopreserved PBMC by using peptide pools corresponding to DNA and MVA vaccine-expressed proteins (Env, Gag, Pol, Nef, Rev and Tat). Characterization of T cell responses was carried out using the intracellular cytokine secretion (ICS) assay.

The antibody responses were estimated using ELISA, Western blot and neutralizing antibody by TZM-bl assay.

Table 1. Trial Design

Group	Study Sites	n=32 (vaccine/ placebo	Months			
			0	1	3	6
A	NARI	6/2	ADVAX 4 mg in 1mL	ADVAX 4 mg in 1mL	TBC-M4 5 X 10^7 pfu in 0.5 mL	TBC-M4 5 X 10^7 pfu in 0.5 mL
	NIRT	6/2				
B	NARI	6/2	TBC-M4 5 X 10^7 pfu in 0.5 mL	TBC-M4 5 X 10^7 pfu in 0.5 mL	-	TBC-M4 5 X 10^7 pfu in 0.5 mL
	NIRT	6/2				

RESULTS

Table 2. IFN-γ ELISPOT responses

	Group A ADVAX matched peptide responses		Group A TBC-M4 matched peptide responses		Group B TBC-M4 matched peptide responses		Placebo Any peptide response	
Overall Response Rate	n	%	n	%	n	%	n	%
Post 1st vaccination**	0/12	0.0%	na	na	6/10	60.0%	1/8	12.5%
Post 2nd vaccination**	3/12	25.0%	na	na	6 /11	54.5%	2/8	25.0%
Post 3rd vaccination***	na	na	12/12	100.0%	11/12	91.7%	3/8	37.5%
Post 4th vaccination***	na	na	12/12	100.0%	na	na	0/4	0.0%
3 months Post 4th vaccination	10/11	90.9%	8/11	72.7%	6/11	54.50%	0/7	0%
6 months Post 4th vaccination	9/12	75%	8/12	66.7%	4/12	33.30%	0/7	0%
3 months Post 4th vaccination	4/10	40%	3/10	30%	4/11	36.40%	0/6	0%

* n = number of positive responders / number of subjects after each vaccination.
** First and second vaccinations consist of ADVAX in Group A and TBC-M4 in Group B
*** Third vaccination consists of TBC-M4 in Groups A and B, while fourth vaccination consists of TBC-M4 in Group A only. A subject was counted as positive if the response was positive at week 1 and/or week 2.

Figure 1. Magnitude of IFN γ responses in trial participants

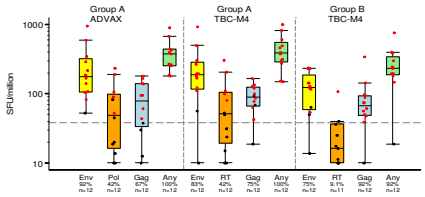


Figure 1. Magnitude of ELISPOT responses at 2 weeks, post last vaccine for Env, Gag and Pol or for any antigen. Black dots = negative and red dots = positive. Box plots represent the median spot forming cells/ million PBMC and the1st and 3rd quartiles. The percent response rate and number of samples included in each plot is shown on the x-axis.

Figure 2. Anti HIV antibodies (frequency and breadth) in trial participants

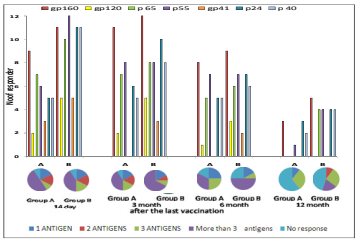


Figure 2. The HIV antibodies were detected at 14 days, 3 months, 6 months and 12 months after the last vaccination using two ELISA kits (ELAVIA and Genetic system, Bio RAD). The samples showing positive response by either of the ELISA kits were further tested by western blot (INNOLIA, Biorad) for determining antigenic specificities of the antibody responses.
The number of volunteers showing bands against different antigens (shown as colored bars) as determined by western blot is shown in the figure on Y axis and time points at which the responses were determined are shown on X axis.
The pie diagrams indicate breadth of antibody responses by volunteers from both the groups as determined by western blot at 14 days, 3 months, 6 months and 12 months after the last vaccination.

Figure 3. Flow Cytometry

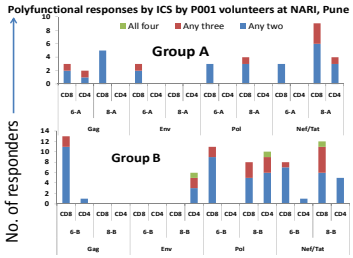


Figure 3. Intracellular cytokine assay was performed at 3 months (6) and 12 months (8) after the last vaccination. The data shown in the figure is from Pune site only. PBMCs were stimulated with pools of different antigens for six hours in the presence of Brefeldin A and were stained by a cocktail of antibodies against different cytokines (IFN-γ, IL-2, TNF-α, MIP1-β) as well as surface markers for CD4 and CD8 after permeabilization. The stained cells were acquired on FACSAria after fixation. The graph shows polyfunctional responses reflecting expression of any two (blue), any three (red) or all four cytokines (green) by the cells as determined by intersecting gates plotted on individual cytokine responses from both the groups. The No. of volunteers showing these responses is plotted on Y axis and antigens against which the responses were seen along with the time points are plotted on X axis.

Figure 4. HIV neutralization

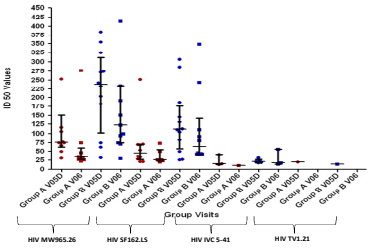


Figure 4. Anti-HIV neutralizing antibodies estimation using TZM-bl assay at 14 days (V05D) and 3 months (V06) after the last vaccination. The samples showing ID 50 above 50 units were considered to be positive. The response was estimated as ID 50 (Y axis) against the panel of tier 1 and tier 2 pseudoviruses. Response by group A volunteers is shown in red colour and that by group B is in blue colour. Circles indicate responses at 14 days and squares indicate responses at 3 months after the last vaccination.

SUMMARY

- No major safety concerns were reported and both vaccine candidates were well tolerated.
- All 12 vaccinees from group A and 11 of 12 vaccinees from group B showed presence of IFN-γ ELISPOT responses response at 14 day following last vaccination
- Nine of 12 vaccinees from group B and 7 of 12 vaccinees from group A showed presence of neutralizing antibody response at 14 days following the last vaccination.
- Neutralizing antibody titers as well as breadth of humoral response as determined by ELISA appeared to be higher in group B volunteers as compared to group A volunteers.
- The cellular immune responses were sustained in 3/10 and 4/11 volunteers in group A and B respectively.
- Amongst the volunteers enrolled at NARI site, secretion of 1-2 cytokines in response to HIV antigens was observed groups A and B. However, polyfunctional responses with expression of all four cytokines were seen in group B only.

CONCLUSIONS

➢ Both DNA and MVA vaccines were found to be safe and were well tolerated.

➢ Heterologous DNA-MVA prime boost strategy elicited comparable T-cell immune responses to the homologous MVA strategy.