

Anti-HIV Antibodies Elicited by Prime Boost Vaccination, vCP1452 + AIDSVAX™ B/B, in Newborns of HIV-infected Mothers

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ABSTRACT

Background
Infants < 72 hours of age born to HIV-1 infected women were enrolled in PACTG 326, a randomized, placebo controlled, double blinded study. The vaccine was a live recombinant Canarypox vCP1452 (ALVAC) (Aventis Pasteur) vaccine which expresses HIV-1 *env*, *gag*, *pol* and *prt* genes with an AIDSVAX™ B/B (B/B) (VaxGen) boost.

Methods
Thirty mother-infant pairs were enrolled into one of 4 cohorts: (1) 1452 alone; (2) 1452 + B/B; (3) saline placebo; and (4) saline placebo and alum placebo. 1452 or saline placebo doses were given at 0, 4, 8, and 12 weeks post birth with the first dose given within 72 hours of birth. Those receiving B/B or alum placebo received them at weeks 8 and 12. Antibodies to gp120, DP31 and Reverse Transcriptase (RT) were measured using qualitative and quantitative validated ELISAs. DP31, a gp41 peptide that is not part of the vaccine, was used as a control for maternal antibody responses. Neutralizing antibodies to HIV-1₉₀ were also measured.

Results (Interim Blinded)
Interim analyses demonstrate that two weeks post the last vaccination (1452 + B/B), 82% of subjects had anti-gp120 responses, whereas only 55% had anti-DP31 responses. By 12 weeks post vaccination (6 months post birth), DP31 antibodies declined to 13%, but the anti-gp120 responses persisted in 100% of subjects with a previous positive response to the vaccine antigen. In addition, Anti-RT antibody responses were present in 37% of subjects at 12 weeks post vaccination. Quantitative ELISA titers and antibody half-life will be part of the unblinded data analysis. The overall antibody response rates are higher in the B/B group as shown in the table below († positive † tested).

Week	vCP1452			vCP1452 + AIDSVAX B/B		
	DP31	GP120	RT	DP31	GP120	RT
0	100% (1/1)†	100% (1/1)†	100% (1/1)†	92% (9/1)†	100% (1/1)†	100% (1/1)†
14	67% (6/9)	78% (7/9)	82% (8/11)	58% (6/11)	82% (8/11)	73% (8/11)
24	50% (5/10)	40% (4/10)	25% (2/8)†	13% (1/8)†	100% (8/8)†	38% (4/11)

In the 1452 + B/B group, of the 7 subjects that were DP31 negative at week 24, 4 were gp120 antibody positive and 43% of those had neutralizing activity.

Conclusion
Antibody responses were present in vaccinated infants and could be differentiated from passive maternal antibodies. These interim blinded data indicate that, in addition to being safe, this combination prime boost strategy, vCP1452 and AIDSVAX B/B, elicited vaccine-directed binding and neutralizing antibodies in infants.

Table UPDATED ABSTRACT TABLE After UNBLINDING

Week	Placebo		vCP1452		vCP1452 + AIDSVAX B/B	
	DP31	GP120	RT	DP31	GP120	RT
0	100% (7/7)	100% (7/7)	100% (7/7)	100% (9/9)	100% (9/9)	100% (8/8)
14	86% (6/7)	71% (5/7)	100% (7/7)	50% (4/8)	75% (6/8)	50% (4/8)
24	71% (5/7)	42% (3/7)	42% (3/7)	33% (3/9)	22% (2/9)	25% (2/8)
52	0% (0/7)	0% (0/7)	14% (1/7)	0% (0/8)	0% (0/8)	67% (4/6)
76	0% (0/7)	0% (0/7)	0% (0/7)	0% (0/7)	0% (0/5)	20% (1/5)
104	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/5)	25% (2/8)

METHODS

ELISA A quantitative assessment of the presence of vaccine specific antibodies was performed on cryopreserved serum/plasma using an optimized and validated ELISA Standard Operating Procedure (SOP) using Good Laboratory Practice (GLP) Guidelines. Six serial dilutions of serum or plasma beginning at 1/50 were tested in duplicate in microtiter plates coated with either purified gp120_{env} (VaxGen, San Francisco, CA), DP31, synthetic gp41 AVERY peptide not present in the vaccine, or Reverse Transcriptase (RT) protein. For each assay, duplicate antigen-containing and non-antigen-containing wells are set up for each serum and two negative and two HIV+ positive control sera were utilized. A score (i.e., O.D. antigen – O.D. non-antigen) with the background subtracted is regarded as positive if it exceeds or equals an O.D. of 0.2. The serum titration optical density values are averaged at each serum dilution and the average is plotted as a function of serum dilution. Titers are defined as the reciprocal serum dilutions that yield 50% maximum binding of the standard positive control replicated on each assay.

Neutralizing Antibody Assay Neutralization was measured as a function of reductions in luciferase reporter gene expression after a single round of virus infection in TZM-bl cells. TZM-bl cells were obtained from the NIH AIDS Research and Reference Reagent Program, as contributed by John Kappes and Xiaoyan Wu. These cells are engineered to express CD4 and CXCR5 and contain integrated reporter genes for firefly luciferase and *E. coli* β -galactosidase under control of an HIV-1 LTR. Briefly, cell free virus (200 TCID50) was incubated with serial dilutions of test samples in triplicate in a total volume of 150 μ l for 1 hr at 37°C in 96-well flat-bottom culture plates. Freshly trypsinized cells (10,000 cells in 100 μ l of growth medium containing 75 μ g/ml DEAE dextran and 2.5 μ M insulin) were added to each well. One set of control wells received cells + virus (virus control) and another set received cells only (background control). After a 48 hr incubation, 100 μ l of cells were transferred to 96-well black solid plates (Costar) for measurements of luminescence using Bright Glo substrate solution as described by the supplier (Promega). Neutralization titers are the dilution at which relative luminescence units (RLU) were reduced by 50% compared to virus control wells after subtraction of background RLU.

Statistical Analyses Rates of positive O.D. were summarized by antigen at each week and compared among treatment groups using Fisher's Exact Test (Figures 1A-1C). Elisa titers were summarized at each week by treatment group using box plots (Figures 2A-2C). In these summaries, Elisa titers <50 with positive OD were considered as equal to 50 and titers <50 with negative OD as equal to 25. Elisa titers were then categorized as follows: >50, <50 with O.D. positive, <50 with O.D. negative. The proportions of subjects in each category were presented by week in Figures 3A-3C and were compared among the treatment groups using Fisher's Exact Test. Neutralizing antibody titers of 10 or higher were considered positive.

BACKGROUND

- Study Design** Phase I/II randomized, placebo controlled double blinded study.
- Vaccine Product** ALVAC vCP1452 from Aventis Pasteur is a recombinant Canarypox vector expressing HIV *env*, *gag*, *pol* and *prt*. AIDSVAX™ B/B is an HIV envelope boost containing HIV-1 gp120_{env} and gp120 *ORF8*.
- Cohort** 30 Mother-Infant pairs (23 Infants received all four doses) were randomized into one of four cohorts: I. vCP1452 alone, II. vCP1452 + AIDSVAX™ B/B, III. Saline Placebo, IV. Saline Placebo + Alum Placebo.
- Analyses** All infants were uninfected. One placebo participant who received two doses was included. The two placebo groups were combined for the analyses. Responses to the HIV antigen, DP31, were used as a control for the presence of passively acquired maternal antibodies.
- Safety and Cellular Studies** ALVAC vCP1452 alone or combined with AIDSVAX™ B/B is safe in infants. Cytotoxic T lymphocyte responses were low and not significantly different between the three groups. ALVAC vCP1452 combined with AIDSVAX™ B/B increased lymphoproliferative responses. See Dr. Betty McFarland's oral presentation, Paper #52, Session 14.

RESULTS

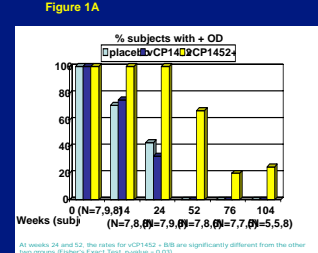


Figure 2A

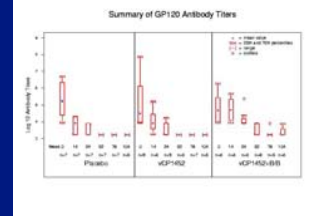


Figure 3A Distribution of Titers for Gp120

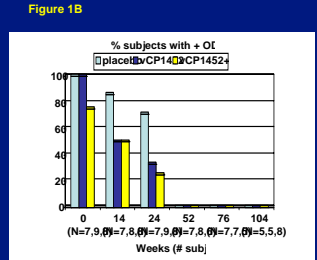


Figure 2B

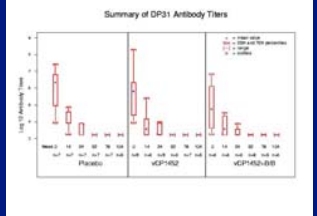


Figure 3B Distribution of Titers for DP31

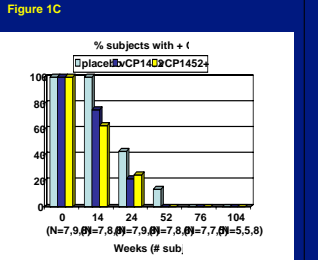


Figure 2C

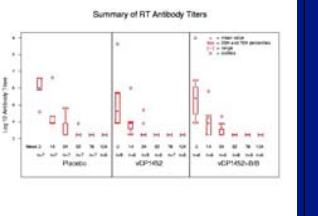


Figure 3C Distribution of Titers for RT

SCHEDULE OF VACCINE AND EVALUATIONS

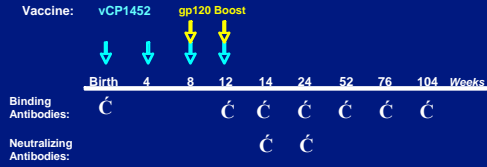
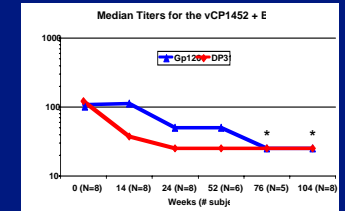


Figure 4



Anti-gp120 responses were detectable in the absence of DP31 responses, but the titer was below the analytical limit of the endpoint determination.

Table 2

Week of Assessment	Binding Antibody (DP31 negative)	Env LPA response (DP31 negative)	Neutralizing Antibody ²
14	4 subjects	75% (3/4)	50% (2/4)
24	6 subjects	83% (5/6)	50% (3/6)
52	4 subjects	75% (3/4)	50% (2/4)
76	1 subject	100% (1/1)	0% (0/4)
104	2 subjects	100% (2/2)	50% (1/2)

¹Env LPA defined as a proliferative response on two or more occasions for study duration. ²Neutralizing Antibody responses measured at week 14 or 24.

CONCLUSIONS

- Antibody responses to HIV-1 envelope were present in the vaccinated infants that received the envelope protein boost and these responses could be differentiated from passive maternal antibodies.
- Anti-gp120 antibody responses were detected in some cases out to week 104.
- Vaccine elicited antibody responses to RT, a component of vCP1452, could not be differentiated from passively acquired maternal antibodies.
- Some of the vaccine-directed binding antibodies were capable of neutralizing activity against HIV-1₉₀.
- The humoral response frequently corresponds with a CD4 proliferative response.
- In addition to being safe in infants, this combination prime boost strategy, vCP1452 and AIDSVAX B/B, elicited vaccine-directed binding antibodies.

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