Phase I/II Randomized Trial of Safety and Immunogenicity of LIPO-5 Alone, ALVAC-HIV (vCP1452) Alone, and ALVAC-HIV (vCP1452) Prime/LIPO-5 Boost in Healthy, HIV-1-Uninfected Adult Participants

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Finding an effective human immunodeficiency virus type 1 (HIV-1) vaccine remains a major global health priority. In a phase I/II, placebo-controlled trial, healthy, HIV-1-negative adults were randomized to receive one of five vaccine regimens: LIPO-5 (combination of 5 lipopeptides) alone (250 μg), ALVAC-HIV (vCP1452) alone, or 3 groups of ALVAC-HIV (vCP1452) followed by ALVAC-HIV (vCP1452) plus LIPO-5 (250, 750, and 2,500 μg). Only 73/174 participants (42%) received all four vaccinations due to a study halt related to myelitis. There were no significant differences in systemic reactions between groups or in local reactogenicity between groups receiving ALVAC-HIV (vCP1452). Significant differences in local reactogenicity occurred between groups receiving LIPO-5 (P = 0.05). Gag and Env antibodies were undetectable by ELISA 2 weeks after the fourth vaccination for all but one recipient. Antibodies to Gag and Env were present in 32% and 24% of recipients of ALVAC-HIV (vCP1452) alone and in 47% and 35% of ALVAC-HIV (vCP1452) + LIPO recipients, respectively. Coadministration of LIPO-5 did not significantly increase the response rate compared to ALVAC-HIV (vCP1452) alone, nor was there a significant relationship between dose and antibody responses among ALVAC-HIV (vCP1452) alone, ALVAC-HIV (vCP1452) alone, or 3 groups of ALVAC-HIV (vCP1452) followed by ALVAC-HIV (vCP1452) plus LIPO-5 (250, 750, and 2,500 μg).

The study described in this paper, HVTN 042/ANRS019, was conducted in France (7–9). The ALVAC-HIV candidate vaccines induced HIV neutralizing antibodies in most vaccine recipients and CTL responses in a subset of vaccine recipients (10–19). This induction occurred with or without a boost regimen using other Sanofi Pasteur (formerly Aventis Pasteur) vaccine candidates or HIV-1 recombinant gp120 vaccines.

Lipopeptide vaccines have been used in animal models (20–24) and were observed to induce simian immunodeficiency virus (SIV)-specific CTLs in macaques (24). Although the responder macaques were not protected against infection with SIV (25, 26).
they showed better control of viremia (27). In further macaque studies, the strength of the CD4+ response has been correlated with induction of a multiepitopic CD8+ response, possibly allowing better control of virus after challenge (28). In various animal species, lipopeptides can elicit or increase various B- and T-cell immune responses where nonacylated peptides or whole proteins had no effect. In one study, a lipopeptide formulation was found to protect chimpanzees against Plasmodium falciparum malaria by immunization with a conserved liver-stage antigen (29). HIV-1 lipopeptide vaccines induced multiepitopic B- and T-cell responses in humans (30).

Four monopalmitoylated lipopeptide vaccines, LIPO-4, LIPO-5, LIPO-6, and LIPO-6T, have been prepared and tested by the Agence Nationale de Recherche sur le Sida (ANRS) alone and in collaboration with Aventis Pasteur (LIPO-5 and LIPO-6T) and Biovector Therapeutics. It was hypothesized that induction of T cell responses could be partially explained by the endocytosis of the lipopeptides into dendritic cells and exogenous protein pathways inducing CD8+ T cells (31) and that combinations of vaccines might induce higher-frequency CD8+ CTL responses than had been attained with individual vaccine candidates.

The NIAID-supported HIV Vaccine Trials Network (HVTN) conducted the current trial (HVTN 042/ANRS019) to evaluate the safety and immunogenicity of LIPO-5 alone and in combination with the canarypox vector, ALVAC-HIV (vCP1452).

**MATERIALS AND METHODS**

The clinical trial is registered on ClinicalTrials.gov with registry number NCT00076063.

**Trial products.** LIPO-5, LIPO-5 placebo, ALVAC-HIV (vCP1452), and placebo-ALVAC and diluents were provided by Aventis Pasteur S.A. (now Sanofi Pasteur).

**LIPO-5 and placebo.** LIPO-5 is a mixture of 5 synthetic lipopeptides in which the sequences represent CTL epitopes within HIV-1 Gag, Pol, and Nef. LIPO-5 was administered at 50, 150, or 500 μg per lipopeptide, i.e., 0.25 (L1), 0.75 (L2), and 2.5 (L3) mg total dose per injection. LIPO-5 placebo (diluent for LIPO-5) is a 20 mM Tris-HCl-3% glucose buffer.

**ALVAC-HIV (vCP1452).** ALVAC-HIV (vCP1452) is a host range-restricted recombinant canarypox virus vector; while it can infect mammalian cells and produce foreign DNA, it does not replicate in mammalian cells (32, 33). The following genes were inserted: the gene for HIV-1 envelope gp120 (strain MN) linked to the transmembrane portion of HIV-1 gp41 (strain LAI), the HIV-1 LAI gag gene (encoding the entire Gag protein), the sequence of a portion of the pol gene (encoding the protease), and the gene for a synthetic polynucleotide encompassing several known human CTL epitopes from the nef and pol gene products. Promoter sequences encoding the E3L and K3L vaccinia virus proteins were inserted in the C6 site in order to enhance the immune responses. The vaccine dose was 10^{7.26} 50% tissue culture infective doses (TCID_{50}). Placebo-ALVAC contained Tris-HCl buffer, virus stabilizer (lactoglutamate), and freeze-drying medium. ALVAC-HIV (vCP1452) and placebo-ALVAC were each supplied as a sterile, lyophilized product in single-dose vials and were reconstituted with 1 ml of saline (0.4% NaCl). The syrings for ALVAC-HIV (vCP1452) and placebo-ALVAC were covered with a blue overlay to maintain the blind.

**Trial design.** The trial was a phase I/II, multicenter, randomized, placebo-controlled, double-blind trial to evaluate the safety and immunogenicity of LIPO-5 alone, ALVAC-HIV (vCP1452) alone, and ALVAC-HIV (vCP1452) (prime) followed by LIPO-5 (boost) (Table 1). Eligible participants were randomly assigned (Fig. 1) to one of 5 groups to receive one of 5 vaccine regimens or placebo administered at months 0, 1, 3, and 6 with follow-up to month 18. The prime-boost arms received one of 3 doses of LIPO-5 (50, 150, or 500 μg of each lipopeptide for total doses of 250, 750, and 2,500 μg per injection). All vaccinations contained 1 ml of fluid and were administered by intramuscular injection into the deltoid muscle. Participants were enrolled concurrently for all arms. The randomization sequence was obtained by computer-generated random numbers. The trial protocol was approved by the respective institutional review boards (IRB) (Saint Louis University IRB, University of California San Francisco Committee on Human Research, Fred Hutchinson Cancer Research Center IRB, The IRB at the University of Alabama at Birmingham, University of Rochester Research Subjects Review Board, Partners IRB, The Miriam Hospital’s Clinical Research Review Board, University of Maryland IRB, Johns Hopkins Committee on Human Research, Columbia University IRB, Vanderbilt University, and Duke University IRB), and all subjects provided written informed consent.

**Eligibility criteria.** Healthy HIV-1-uninfected adults, ages 18 to 50 years, in good general health without clinically significant medical histories or physical findings and with acceptable white
blood cell count, hemoglobin, and renal and liver function were eligible. Subjects were excluded if they had a history of uveitis or uveitis-associated conditions (chronic Lyme disease, active mycobacterial diseases, and/or sarcoidosis).

Safety assessment. Local and systemic reactogenicity was evaluated for 3 days after each injection, and adverse events were followed for 18 months after the first injection. In a study of a different lipopeptide HIV vaccine in individuals who previously received a different ALVAC-HIV vaccine, 2 subjects developed symptoms consistent with anterior uveitis. Therefore, all participants in HVTN 042 were questioned at each visit about any ocular symptoms that could be consistent with uveitis, including eye pain, redness, photophobia, and visual changes. In addition, participants who developed eye symptoms between visits were instructed to contact the site. All participants reporting ocular symptoms were evaluated further by the site clinician and promptly referred for a slit lamp exam as appropriate to rule out uveitis. Solicited reactogenicity included local injection site pain, tenderness, erythema and induration, fever, chills, myalgia, arthralgia, headache, malaise and/or fatigue, nausea, and vomiting. Laboratory assessments were performed 2 weeks after the second, third, and fourth vaccinations and at the last visit on days 42, 98, 182, and 546 and included alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, complete blood count with differential, and urinalysis (protein, hemoglobin, and glucose).

Immunogenicity assessment. Immunogenicity endpoints included HIV-1-specific CD8+ T-cell responses measured by gamma interferon (IFN-γ) enzyme-linked immunosorbent spot assay (ELISpot) and antibody responses against HIV Env and Gag measured by enzyme-linked immunosorbent assay (ELISA). The ELISpot assay was standardized and optimized in the HVTN cen-
tral lab in order to define T cell responses as potential correlates of immunity (34).

HIV-specific binding antibody. Anti-Gag and anti-Env antibody responses were determined by a validated ELISA, as previously described (35, 36). Sera (1:100) were tested in duplicate on microtiter plates (NUNC) coated with purified p24 Gag (Quality Biological Inc.) or MN gp120 (Protein Sciences, Meriden, CT). Standard curves were generated from the plot of absorbance (450 nm) against the log of serum dilution, and sigmoidal curves were fit using a four-parameter logistic equation (Softmax Pro). A positive response was defined by the optical density (OD) of antigen-containing wells minus the OD of non-antigen-containing wells having a value of >0.2.

HIV-specific IFN-γ ELISpot. The IFN-γ ELISpot kit (Becton Dickinson, San Jose, CA) was used according to the manufacturer’s directions and was previously described (37). Sets of 15-amino-acid peptides overlapping by 11 residues were synthesized, covering the sequences of BRU Nef, HXB2 Pol, and Env Con B by SynPep (SynPep, Dublin, CA). These peptides were combined into pools containing up to 85 peptides and were validated for low false positivity against a set of 50 HIV-1-seronegative donor samples. Additional peptides obtained through the AIDS Reagent Program and corresponding to selected sequences within Gag, Pol, and Nef that matched the vaccine constructs were provided by the ANRS and pooled at the Fred Hutchinson Cancer Research Center (FHCRC) central laboratory.

Statistical considerations. (i) Sample size. Sample size calculations are based on 30 vaccinees per group. Within the prime-boost regimens, the study aimed to select the LIPO-5 dose group that gave the highest probability of a positive cellular immune response and to prefer lower-dose groups that provided immune response probabilities comparable to those in higher dose groups. The trial had at least 80% probability of selecting the best dose group (the one with the highest response probability) if its response probability was at least 0.11 to 0.15 greater than the second best dose group (e.g., response probabilities of 0.21 versus 0.10 or 0.45 versus 0.30).

For the objective of showing noninferiority of a lower dose group compared to a higher dose group, the trial had 80% power (1-sided 0.05-level test) to demonstrate that the difference in response probabilities was less than 0.30, if the response probabilities were equal in the two groups being compared. For the objective of showing superiority of one group over another, the trial had 80% power (2-sided 0.05-level test) to detect a pairwise arm difference in response rates of 0.10 versus 0.44, 0.20 versus 0.58, 0.30 versus 0.69, 0.40 versus 0.78, and 0.50 versus 0.86.

(ii) Statistical analysis. All data from enrolled participants who received at least one vaccination were analyzed. Analyses were performed using SAS and R statistical software. Descriptive statistics, t tests, and Fisher's exact tests were used to compare baseline characteristics among study groups.

(iii) Reactogenicity. The number and percentage of participants experiencing each type of reactogenicity sign or symptom were tabulated by severity and vaccine regimen. For a given systemic sign or symptom, each participant's reactogenicity was counted once under the maximum severity for all injection visits. For a given local sign or symptom, each participant's reaction was counted once under the maximum severity for (i) all injection visits providing ALVAC-HIV (vCP1452) alone and (ii) for all injection visits providing either LIPO-5 alone or ALVAC-HIV (vCP1452)+LIPO. For each reactogenicity event type, the Kruskal-Wallis rank sum test was used to test for overall differences between groups after converting each outcome to an integer variable ranging from 1 to n, where n is the number of event categories. The placebo groups were pooled to provide more power for comparing vaccine groups to placebo.

(iv) Immunogenicity. Rates of positive responses for ELISA and ELISpot were estimated for each group and time point (days 0, 98, and 182), where the statistical procedures for defining positive responses are described below (37). For ELISA, the response rates were computed for p24 (Gag) and MN gp120 (Env). For ELISpot, the response rates were computed for each of 12 peptide pools separately (ANRS Gag, Nef, and Pol; consensus B Gag pools 1 and 2; Env pools 1, 2, and 3; FHCRC Nef and FHCRC Pol pools 1, 2, and 3) as well as for response to any of the 12 pools. For each time point, positive response rates were compared between groups using Fisher’s exact test. Two-sided 95% confidence intervals about positive response rates were calculated using the Wilson method (38). All P values are two sided, and results are considered statistically significant if P was ≤0.05.

(v) IFN-γ ELISpot assay. To determine a positive response to a specific peptide pool, a permutation test was applied to each of the antigen-specific responses versus negative-control responses using the Westfall-Young approach performed at a 0.05 level to adjust for the multiple comparisons. A variance filter for the antigen-specific responses was used: samples with a ratio of experimental- to control-variance to (median + 1) that exceeded 100 were discarded from the analysis. Phytohemagglutinin (PHA) was used as the nonspecific positive control.

RESULTS

Participants and characteristics. A total of 174 participants were enrolled between 1 April 2004 and 30 June 2004 at 12 U.S. sites. Of these, 101 (58%) were males. The median age was 31 years (range, 18 to 50), and 119 (68%) were white. Vaccinations were halted due to a serious adverse event described below. Prior to the trial halt, 174 (100%), 168 (97%), 142 (82%), and 73 (42%) of the subjects received injections at months 0, 1, 3, and 6, respectively.

Safety results. No statistically significant differences in baseline demographic variables (Table 2) or in systemic reactions (Fig. 2A) were observed between groups (P > 0.05). For local injection site reactogenicity in groups receiving the ALVAC-HIV (vCP1452) injections, no significant differences between groups were observed for pain, tenderness, erythema, and induration (P > 0.05) (Fig. 2B). For local reactogenicity in groups receiving the LIPO-5 injections, significant dose-related differences between groups were observed for pain, erythema, and induration (P ≤ 0.05) but not for tenderness (P > 0.05) (Fig. 2C).

A total of 157 participants reported at least one adverse event (AE). For participants reporting multiple AEs, the maximum severity and relationship to study product were counted when the number of participants experiencing one or more AE was tabulated. For 72, 66, 16, and 3 participants, the maximum severity of their AE was assessed as mild, moderate, severe, and life-threatening, respectively. The maximum relationship of AE to study product among 16, 3, and 33 participants was definitely, probably, and possibly related to vaccine, respectively. Seven women became pregnant during the study; one woman had 2 pregnancies. The outcomes of the pregnancies included 6 live births without known fetal complications, 1 elective abortion, and 1 unknown.
Thirteen serious adverse events occurred in 10 participants. Five, 2, 2, and 4 were considered definitely, possibly, probably, and not related, respectively. One event each was considered moderate (Bell’s palsy, not related) and life-threatening (myelitis, possibly related) and 11 events were considered severe. Of the 11 severe events, 5 were considered definitely related (injection site pain, 2 events in the same person), 1 possibly related (fatigue), 2 probably related (headache and nausea in the same person), and 3 not related (transient ischemic attack, asthmatic bronchitis, and migraine) to the vaccine. A moderate event of Bell’s palsy in a placebo recipient was not related to vaccine. One participant had myelitis with gait and sensory changes (graded as life-threatening and possibly related) and one LIPO recipient but were present in 32% (8/25) and 24% (6/25) of recipients of ALVAC-HIV (vCP1452) alone, respectively, and present in 47% (39/82) and 35% (29/82) for the ALVAC-HIV (vCP1452)+LIPO arms combined (Fig. 3). The ALVAC-HIV (vCP1452)+LIPO arms combined did not significantly increase the antibody response rate compared to ALVAC-HIV (vCP1452) alone (P = 0.25 and P = 0.34). There was not a statistically significant dose response in antibody responses for the ALVAC-HIV (vCP1452)+LIPO arms as measured by response rate (P = 0.96 and P = 0.10).

**IFN-γ ELISpot assay.** Over 90% of study participants had no positive IFN-γ ELISpot response to any peptide pool at any time point (Fig. 4A and B). The rates of positive responses to any peptide pool were 2/28 (7.1%), 1/25 (4.0%), and 2/23 (8.7%) at days 0, 98, and 182 for the pooled placebo group and 3/134 (2.2%), 10/121 (9.1%), and 6/123 (4.9%) at days 0, 98, and 182 for the pooled vaccine arms. Of all samples, ≥99% had a positive response to the nonspecific positive control, PHA (data not shown). There was no evidence that any of the vaccine regimens induced positive ELISpot responses to any target.

For the day 182 ELISpot, 2/23 in the placebo group responded (one responded to 2 antigens, hence the 3 dark points shown in Fig. 4B), while 6 of 123 in the active treatment groups had a response. At day 182, the net positive response rate (subtracting the value for the combined placebo groups) was −3.8%, with a 95% confidence interval (95% CI) of −22.2 to 4.5%; therefore, this

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### TABLE 2 Demographics and vaccination frequencies

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<sup>a</sup>No significant differences were noted using a two-sided Fisher’s exact test for categorical variables and a two-sided t test for the distribution of age. LIPO-5 was given at 50, 150, or 500 μg per lipopeptide, for total doses of 0.25, 0.75, and 2.5 mg per injection. Group A received 500 μg LIPO-5 at months 0, 1, 3, and 6 intramuscularly (IM); group B received ALVAC HIV-1 at months 0, 1, 3, and 6 IM; group C received ALVAC HIV-1 at months 0, 1, 3, and 6 plus 50 μg LIPO-5 at months 3 and 6 IM; group D received ALVAC HIV-1 at months 0, 1, 3, and 6 plus 150 μg LIPO-5 at months 3 and 6 IM; group E received ALVAC HIV-1 at months 0, 1, 3, and 6 plus 500 μg LIPO-5 at months 3 and 6 IM.

<sup>b</sup>Combined placebo recipients for all groups, i.e., placebo-ALVAC and LIPO-5 placebo.
FIG 2 Reactogenicity. (A) Maximum systemic reactogenicity by the treatment group; (B) maximum local reactogenicity by the treatment group for the ALVAC-HIV injections; (C) maximum local reactogenicity by the treatment group for the LIPO-5 injections. Each bar chart shows the percentage of volunteers that had a maximum reactogenicity event by severity and type. P1 and P2, placebo recipients from the groups receiving LIPO-5 alone and ALVAC alone, respectively. P3 to P5, placebo recipients from the groups receiving ALVAC-HIV plus LIPO (L1 to L3) described below. Vaccine recipients in group L received LIPO-5 alone, those in group A received ALVAC alone, and those in group A+L received ALVAC-HIV plus LIPO-5 (250 µg). Group A+L received ALVAC-HIV plus LIPO-5 (750 µg), and group A+L2 received ALVAC-HIV plus LIPO-5 (2,500 µg).
study had precision to rule out vaccine-induced positive response rates larger than 4.5%.

**DISCUSSION**

Despite growing efforts and recent advances (39–41), practical strategies for HIV prevention remain challenging to develop and implement. Although vaccines have historically been among the most powerful tools for advancing public health, affordable and effective HIV vaccines remain unavailable.

This study was done to evaluate the potential benefit of a LIPO-5 product in boosting immunogenicity to an ALVAC-HIV-based vaccine, but vaccinations were discontinued early due to safety concerns raised by a case of myelitis that was assessed as possibly related to LIPO-5 vaccine. A rigorous clinical evaluation did not reveal an alternative etiology for the myelitis. We are not aware of other cases of myelitis associated with receipt of lipopeptide products. At the time of discontinuation of the study, a review of safety data for 248 participants receiving lipopeptide vaccine products in 10 ANRS-sponsored trials found no similar demyelination event (42). Safety data from 200 healthy HIV-uninfected subjects (ages 21 to 55 years) and 48 HIV-infected subjects (ages 21 and over) were reviewed. The majority of the participants were males. Twelve serious adverse events occurred in nine HIV-uninfected subjects; of these, two (fever and anterior uveitis) were considered related to the vaccine. Sixteen serious adverse events occurred in HIV-infected participants, including a case of aggravated sciatica.

Additionally, although there was close monitoring for anterior uveitis in our study, no cases were found. Subjects in another trial who had previously received a different ALVAC-HIV vaccine and later received 3 and 2 doses of LIPO-6 with QS21 were diagnosed with anterior uveitis at 4 months and 1 year after the last vaccination, respectively. The second case of uveitis was not included in the safety analysis, as it occurred after the study ended (42). Based on these findings, the FDA requested that the protocol informed consent form for this study be revised to describe the neurologic adverse events associated with the lipopeptide antibiotic daptomycin (Cubicin). The Cubicin package insert listed decreases in nerve conduction velocity (also seen in comparators) and adverse events (e.g., paresthesias and Bell’s palsy) possibly reflective of peripheral (also seen in animal studies) or cranial neuropathy. However, when the case of myelitis appeared to be disabling, all study vaccinations were permanently discontinued. Unfortunately, as with many adverse events, the myelitis case remains unexplained. More recently, the safety of the LIPO-5 vaccine was evaluated in healthy volunteers enrolled in the ANRS Vac 18 phase II trial, and no safety concerns were noted (43).

Prophylactic and therapeutic vaccine trials testing LIPO-5 in combination with DNA or modified vaccinia Ankara HIV are ongoing in France.

The lipopeptide vaccine in our hands was minimally immunogenic. Previous reports demonstrated that anti-HIV antibody responses could be elicited against ALVAC-HIV (vCP1452) alone in the absence of a protein boost (6). Here, we confirm that ALVAC-HIV (vCP1452) elicits modest levels of anti-HIV antibodies and we show that the coadministration of LIPO-5 at the doses tested did not increase titers of antibody to gp120 Env and p24 Gag. LIPO-5 administered in another trial (43) showed more robust results. CD8+ T cell responses to at least one pool of HIV-1 peptides ranged from 62% to 69% in individuals receiving dosages of LIPO-5 similar to those in this trial (43). Reasons for the differences in the immune responses between the two studies may be in

**FIG 3** ELISA. For each antigen, the background-adjusted optical density (OD) at a dilution of 1:100 for each antigen, by treatment arm, for all vaccinees (by group) at day 182 and the background-adjusted OD for all participants at day 0 together with all placebo recipients combined are shown. The numbers and percentages of positive responders over the number of assays tested are provided at the top of the figure. Positive responders (OD > 0.2) are indicated by black points and negative responders by gray points. Group L received LIPO-5 alone, group A received ALVAC-HIV alone, group A+L1 received ALVAC-HIV plus LIPO-5 (250 μg), group A+L2 received ALVAC-HIV plus LIPO-5 (750 μg), and group A+L3 received ALVAC-HIV plus LIPO-5 (2,500 μg). Mp24, p24 Gag; PS-gp120, MNgp120.
part due to the different assays used to assess responses or the differences in the ALVAC vectors.

The published data on the same construct, ALVAC-HIV (vCP1452) alone, in HVTN 203 (6) and other studies are consistent with the HVTN 042 findings. The ALVAC used in RV144 was a different vaccine, ALVAC-HIV (vCP1521) given with AIDSVAX B/E. Despite modest cellular responses to the combination regimen as determined by ELISpot (19.7% of vaccine recipients re-

![Fig 4](https://example.com/fig4.png)

**FIG 4** IFN-γ ELISpot assay. The background-corrected response to each antigen for all vaccinees (combined into one group) at days 98 (A) and 182 (B) and the background-corrected responses for all participants at day 0 plus all placebo recipients combined are shown. The numbers and percentages of positive responders over the number of assays tested are provided at the top of the figure. Positive responders are indicated by black points and negative responders by gray points. The negative control consists of 6 wells of sample PBMC in medium with no peptide stimulation. ≥99% of samples had a positive response to the nonspecific positive control, PHA (data not shown).
sponded to Gag or Env, compared to 7.3% of placebo recipients), the vaccine was protective. The results of an earlier phase II study of ALVAC-HIV (vCP1452) alone and in combination with rgp120 (AIDS/VAX B/B) (6) were a negative trigger for a phase III correlates trial. For the group receiving ALVAC-HIV (vCP1452) alone, the net cumulative CD8+ ELISpot response was only 13% (95% CI, −1% to 26%) and the net cumulative CD4+ ELISpot response was 1% (−7% to 9%). These disappointing CD8+ T-cell response rates were significantly lower than expected based on the responses observed with earlier-generation canarypox products, such as vCP205. Another multisite international trial in the Caribbean and South America evaluated ALVAC-HIV (vCP1452) with and without boosting with MN rgp120 (44). In that study, the ELISpot response for the ALVAC-HIV (vCP1452)-alone group was only 2%.

Conclusions. Vaccinations were halted due to a safety concern based on a case of myelitis that was assessed as possibly related to LIPO-5 vaccine. A safety review of ANRS lipopeptide vaccines was conducted by others (42), and the case of myelitis remains an isolated event. In general, there was no appreciable cell-mediated immunity detected in response to the vaccines used in this study, and antibody responses were limited. Although ALVAC-HIV (vCP1452) alone was modestly immunogenic, the coadministration of LIPO-5 did not provide any additional immunogenicity.

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REFERENCES


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