Safety and Immunogenicity of Combinations of Recombinant Subtype E and B Human Immunodeficiency Virus Type 1 Envelope Glycoprotein 120 Vaccines in Healthy Thai Adults

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Safety and immunogenicity of 2 recombinant human immunodeficiency virus (HIV) type 1 envelope glycoprotein (gp) 120 vaccines derived from SF2 (subtype B) and CM235 (CRF01_AE, Thai E) were evaluated in 370 Thai adults at low risk of HIV infection. Various doses of CM235 (25, 50, or 100 μg) and SF2 (0, 25, or 50 μg) gp120 were used. Eighty volunteers received placebo. There were no serious adverse events related to vaccination. Binding antibody developed in all vaccine recipients. There was no dose response to CM235 gp120, but a dose response to gp120 SF2 was present. Neutralizing antibodies to subtype E HIV-1 NPO3 and subtype B HIV-1 SF2 developed in 84% and 82% of vaccine recipients, respectively. Lymphoproliferative responses were detected in >95% of vaccine recipients. There was no evidence of antigenic interference in HIV-specific humoral or cellular responses. The gp120 Thai E and SF2 vaccines were safe and immunogenic in combination and could be advanced into phase 3 testing.

AIDS has emerged as an uncontrolled, worldwide public health problem and is associated with high morbidity and mortality. From the beginning of the pandemic until the end of 2001, >40 million people worldwide were infected with human immunodeficiency virus (HIV) [1]. The prevalence of adult HIV infection in Thailand is estimated to be 2.15%; ∼1 million persons have been infected [1], despite an effective condom campaign [2, 3]. An HIV preventive vaccine is urgently needed as a supplementary public health tool against this pandemic.

One potential obstacle to the development of an effective HIV vaccine is genetic variation, particularly in the envelope protein [4]. HIV genetic sequences (genotypes) form genetic clusters or subtypes, some of which are geographically limited. Envelope gp160 amino acid sequence variation between genetic subtypes is ∼20%–30% [5, 6]. Given this degree of intersubtype variation, matching the genetic subtype of the vaccine with the predominant circulating genotype has been one approach to selection of candidate vaccines for eventual phase 3 trials. In Thailand, the principal
variant of HIV is a recombinant of subtypes A and E, designated CRF01_AE (referred to subsequently as subtype E) [5–9].

A number of HIV-1 envelope (gp120/gp160) vaccines have been tested in phase 1 and 2 trials in HIV–1-seronegative volunteers [10–19], and 2 subtype B gp120 subunit vaccines (MN- and SF2-based) have been tested in Thailand [20, 21]. The HIV gp120 subunit vaccines evaluated in human clinical trials in Thailand were derived from T cell line–adapted (TCLA) strains of HIV-1 that use the CXCR4 (X4) coreceptor. Despite the induction of high titer of neutralizing antibody (NAb) against homologous and some heterologous TCLA strains, primary X4 and CCR5 (R5) HIV-1 strains were not neutralized [22, 23]. The failure of TCLA gp120 vaccines to generate primary isolate NAb has been attributed to the lack of appropriate quaternary structure of the gp120, the lack of induction of conformation-dependent antibody, masking of immune epitopes within the gp120 of primary isolates, and/or the immunodominance of variable linear epitopes such as the V3 loop [24–26].

Animal studies using the subtype E, CM235 gp120 vaccine with MF59 adjuvant showed high-titer, HIV-specific antibody that preferentially recognized conformationally intact epitopes. Although the NAb response to CM235 gp120 was capable of neutralizing heterologous, subtype E TCLA HIV-1 strains, there was little neutralization of homologous CM235 in peripheral blood mononuclear cells (PBMCs), similar to the strain-specific neutralization seen in nonhuman primates [27]. Vaccination of rabbits with a subtype E, R5-using HIV-1 gp120 (CM244) resulted in high-titer CM244-specific antibody that neutralized CM244 but not heterologous subtype E strains [28]. Given the predominance of subtype E infection in the region, Thai national authorities approved human clinical trials of 2 different subtype E vaccines, CM235 gp120 and CM244 gp120. Here, we describe a phase 2 trial of the safety and immunogenicity of subtype E CM235 gp120, alone and in combination with subtype BSF2 gp120, in low-risk, HIV-seronegative Thai adults.

**SUBJECTS AND METHODS**

**Study Design**

Following an open-label, dose-escalation study of 12 volunteers (J.K., unpublished data), a randomized, double-blind, placebo-controlled, multicenter trial (phase 2) was conducted by the Thai AIDS Vaccine Evaluation Group (TAVEG) at 4 collaborating centers: Thai and US Components of the Armed Forces Research Institute of Medical Sciences (Bangkok); Faculty of Medicine, Siriraj Hospital, Mahidol University (Bangkok); Vaccine Trial Centre, Faculty of Tropical Medicine, Mahidol University (Bangkok); and Research Institute for Health Sciences, Chiang Mai University (Chiang Mai). Volunteers in the phase 2 trial were healthy HIV-seronegative adults (20–50 years old, of both sexes) from the community, who denied engaging in high-risk behavior for HIV exposure. All volunteers were counseled regarding HIV risk behavior and were tested for HIV before enrollment and 5 times during the trial. HIV status was based on screening with an HIV-specific EIA (Vironostika HIV-Uni-Form II plus O; Organon Teknika), and EIA-positive results were further evaluated with an HIV-specific Western blot assay (New LAV blot I; Bio-Rad Laboratories).

Volunteers were randomly assigned to 1 of 10 groups receiving various dose combinations of CM235 gp120 antigen, SF2 gp120 antigen, and placebo. After passing a test of understanding during a screening protocol before enrollment in this study, all volunteers gave written informed consent. Human experimentation guidelines of authors’ institutions were followed in the conduct of the clinical research reported here. Female volunteers were tested for pregnancy before each immunization. There were no pregnancies detected during the study period.

**Investigational Vaccines**

Two investigational vaccines (CM235 gp120 and SF2 gp120) were produced in genetically engineered CHO cells at Chiron Vaccines (Emeryville, CA). The 2 vaccines were used with the adjuvant MF59, which is also a component of a licensed influenza vaccine. On the basis of prior experiments with monovalent SF2/MF59, doses of 25, 50, and 100 μg were chosen for CM235/MF59. Doses of each antigen (CM235 and SF2) were assessed in a matrix to identify the optimal combination. The response of Thai adults to SF2 gp120 alone has been reported elsewhere [20]. The 4 collaborating centers evaluated CM235 gp120/MF59 vaccine at 25, 50, and 100 μg alone, or combined with the SF2 gp120 vaccine at doses of 25 or 50 μg. Ninety-two volunteers at each center were randomly assigned to receive 1 of the vaccine combinations or placebo; 8 volunteers each were randomly assigned to 1 of 9 vaccine groups (n = 72) or to placebo (n = 20). The placebo contained MF59 adjuvant without immunogen. Volunteers were immunized at study months 0, 1, and 6. Study personnel and volunteers were blinded to the assignment of active agent or placebo.

**Evaluation of Volunteers and Follow-Up**

All volunteers were observed for local and systemic reactions in the 30 min immediately after immunization and were monitored for the next 7 days by use of self-reported diary cards. Each volunteer was contacted 24–48 h after immunization by a study nurse. Clinical evaluation, hematologic studies, serum chemistry measurements, liver and renal function tests, serum for antibody assays, and cells for lymphoproliferation studies were obtained during the course of the study.
Cells and Viruses

**T cell lines.** The CD4–CXCR4+ T cell line H9 was used for all screening and titration NAb assays using the NP03 (X4-tropic, subtype E) and SF2 (X4, subtype B) TCLA HIV strains, as reported elsewhere [20, 22, 29]. The CD4–CXCR4+CCR5+ T cell line A3R5-6 was used for assays in which the CM235M4-C4.6 virus (R5-tropic, subtype E) was tested. A3R5-6 is derived from A3.01 cells and is the result of transfection of an expression plasmid containing CCR5-neo into A3.01 with serial selection by fluorescence-activated cell sorting and genetin for high expression of CD4 and CCR5. Levels of CD4, CXCR4, and CCR5 expression are consistent with levels seen in phytohemagglutinin (PHA)/interleukin (IL)–2–activated PBMCs (J.K., unpublished data). PHA/IL-2–stimulated PBMCs were used for NAb experiments involving primary CM235 virus (R5, subtype E).

**HIV-1 isolates.** As described elsewhere [27], NP03 is a TCLA subtype E virus (X4, E) derived from a participant in the Thai Natural History Study. SF2 is a TCLA subtype B (X4, B) virus similar to the strain used in the gp120 bivalent vaccine. CM235M4-C4.6 is a subtype E, CCR5-tropic virus that initially had been grown in MOLT-4 cells and was further adapted for growth in the A3R5-6 T cell line. It is completely identical in V3 sequence to CM235. It will not infect CCR5+/- PBMCs and is relatively resistant to neutralization by soluble CD4 (J.K., unpublished data). CM235 is a PBMC-derived, early passage, primary HIV-1 isolate and is the parent strain of the subtype E gp120 envelope in the bivalent vaccine tested in this study.

Immunological Assessments

**Binding antibody (BAb) assays.** Anti-gp120 BAb titers were determined by ELISA for each volunteer. Serum samples were tested for BAb at 4 time points: baseline and at 1 month after the first, second, and third immunizations. Serial 3-fold dilutions of serum samples, ranging from 1:100 to 1:218,700, were added to microtiter plates precoated with recombinant gp120/CM235 or gp120/SF2 produced in CHO cells (Chiron Vaccines) at a concentration of 0.2 µg/well. Plates were incubated for 1 h at 37°C and washed, and anti-gp120 antibodies were detected using peroxidase-conjugated goat anti–human IgG (Biosource International), followed by reaction with tetramethyl benzidine substrate. All serum samples were tested on duplicate plates. The optical density was determined at 450 nm and 650 nm (background), and the result was calculated as the difference. Anti-gp120 BAb titer was defined as the reciprocal of the serum dilution that yielded an OD of 1.0, as determined by a third-order polynomial curve fit. Validity of the assay was monitored using a 2-fold dilution of a standard HIV-positive serum, which had to give a titer of 50% of the undiluted standard, and a difference between duplicate samples, which had to be <2-fold. Data are expressed as the geometric mean titer (GMT) of the reciprocal serum dilution. Serum samples were considered to be positive for HIV-specific BAb if the reciprocal titer was ≥100. Nonreactive serum samples were assigned a titer of 50 (half the reciprocal titer of the lower limit of detection of the assay).

**NAb assays.** T cell line–based and PBMC-based neutralization assays were performed as described elsewhere [20, 22, 29]. First, screening assays for NAb to the heterologous TCLA subtype E strain NP03 (X4) and the homologous TCLA subtype B strain SF2 (X4) were performed. Subsequently, NAb responses of a subgroup of volunteers who received 25, 50, or 100 µg of Thai E plus 50 µg of SF2 (n = 95) were analyzed in greater detail. In this subgroup, all volunteers were tested for NAb against homologous CM235 and against CM235M4-C4.6 in A3R5-6 cells. Finally, postvaccination serum samples from this subgroup were serially diluted, and NAb titers against NP03 were determined.

**Screening neutralization assay.** A screening neutralization assay was performed by incubating a fixed dilution (1:10) of pre- and postvaccination serum samples with virus in triplicate wells for 30 min at 37°C. For TCLA isolates, 1 × 10⁵ H9 or A3R5-6 cells were added and incubated overnight. For primary CM235, 1.5 × 10⁶ PHA/IL-2–stimulated PBMCs were added and incubated overnight. The next day, the cells were washed thrice with complete medium. On the day when regular medium rows were >10 ng/mL supernatant was harvested, and p24 analyses were performed. The percentage of neutralization was calculated as follows: 100 × (1 – V_out/V_pre), where V_out is the p24 level in cultures incubated with serum samples taken after the third vaccination, and V_pre is the p24 level in cultures incubated with prevaccination serum samples. Neutralization ≥50% was considered to be a positive NAb response in the assay [20]. For determination of NAb, the samples were assayed twice, when feasible, and the average percentage of neutralization of the 2 assays was calculated. Eighty-five percent of samples had 2 separate determinations, with priority being given to subtype E NP03 assays (nearly 100% were run twice).

**Determination of neutralization titer.** H9 cells were suspended in complete medium at 3.0 × 10⁵ cells/mL. A frozen aliquot of test serum was thawed quickly at 37°C in a water bath. Serum dilutions were set up in a 25-µL volume. Pretitered HIV stock was diluted to 100 TCID₅₀/25 µL of complete medium. Twenty-five microliters of diluted virus stock was added into each of 3 wells of a 96-well box. The virus and serum were allowed to incubate at 37°C for 30 min. H9 cells (1 × 10⁵) were added and incubated overnight. The next day, the cells were washed thrice with complete medium. After the final wash, 200 µL of complete medium was added to each well, and the entire mix was transferred to a round-bottom, 96-well plate. One hundred microliters was removed for analysis and replaced with 100 µL of complete medium on sequential days, starting at day 3. At the point when 10 ng of p24 was detected in pooled,
RESULTS

A total of 370 volunteers were enrolled in this phase 2 trial. The median age was 30.9 years, with males predominant. The most common single occupation was Buddhist monk (20%); education levels were equal to or higher than secondary school for 68% of recruited volunteers. There were no significant differences in age, sex distribution, and education levels among the groups.

Safety profile. The immunizations were well tolerated (table 1). All participants, except 2 subjects in the 50-μg E antigen/0-μg B antigen (50/0) group, reported pain on injection. The pain was rated severe by 1 or 2 subjects (3%–6%) in each group except in the placebo group, in which 6 subjects (8%) rated the pain as severe. The most frequently reported local reaction was pain, occurring in 70% (50-μg E antigen/25-μg B antigen [50/25] and 25-μg E antigen/50-μg B antigen [25/50] groups) to 89% (100-μg E antigen/50-μg B antigen [100/50] group) of subjects. Pain on injection did not increase in frequency with subsequent immunizations. Adverse events possibly, probably, or definitely related to vaccination occurred in 11% of subjects receiving placebo, 19% of subjects receiving HIV gp120 E alone, and in 19% of subjects receiving both antigens. Adverse events possibly, probably, or definitely related to vaccination occurred in 11% of subjects receiving placebo, 19% of subjects receiving HIV gp120 E alone, and in 19% of subjects receiving both antigens. Adverse events possibly, probably, or definitely related to vaccination that occurred in >5 subjects were found only in groups receiving both antigens and included headache (6 subjects [3%]), injection-site edema (6 subjects [3%]), injection-site reaction (11 subjects [6%]), and myalgia (6 subjects [3%]).

One death, caused by suicide, occurred during the trial (placebo group). Eleven other subjects experienced serious adverse events, the majority of which were due to motorcycle accidents. None of these events was considered to be related to the study vaccines, and none resulted in premature withdrawal from the study. There were no significant differences in distribution of reported reactogenicity among the groups. There were no intercurrent HIV infections of volunteers during the study.

Immunological profiles. Among vaccinated volunteers, 15% developed anti-HIV reactivity, as measured by the commercial diagnostic EIA, most frequently detected in the serum samples collected 12 weeks after the third immunization. This EIA reactivity decreased to 1.7% by 6 months after the third immunization. None of these EIA-reactive specimens were confirmed as HIV positive by Western blot.

BAb responses. Vaccination induced BAb to CM235 gp120 or SF2 gp120 or both in all recipients. No placebo recipients had detectable BAb. BAb was observed as early as after the first immunization, increasing to maximum titers after the third immunization. The GMTs after the second and third immunizations were 1449–7764 for CM235 gp120 and 56–2600 for gp120 SF2 (figure 1 and figure 2). No dose response was observed for CM235 gp120 (figure 1). In contrast, there was a clear increase in SF2 gp120 BAb titer with increasing SF2 gp120

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Statistical Analysis

Qualitative demographic data are summarized as percentages and were compared by χ² test; for marital status, “widowed” and “separated/divorced” were combined. Mean ages of different groups were compared by analysis of variance (ANOVA). For reactogenicity and adverse events, assessments were categorized as mild, moderate, or severe and as local or systemic. Differences between vaccine groups, with respect to proportion of subjects exhibiting moderate or severe reactions or adverse events, were analyzed by χ² test.

Immunogenicity data were summarized by percentage of detectable responses and by geometric mean titers (GMTs). For samples with undetectable responses, half of the lower limit of detection of the assay was assigned. Differences in percentage response were analyzed by χ² test, whereas GMTs were analyzed by 2-way ANOVA linear model. We considered differences to be significant at P < .05 (2-sided).

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Table 1. Subjects (n = 383) with postimmunization reactions (within 7 days of any injection) during a phase 1/2 trial of human immunodeficiency virus subtype B and E vaccines in Thailand, by dose group.

<table>
<thead>
<tr>
<th>Reactions</th>
<th>E antigen dose/B antigen dose, µg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25/0 (n = 35)</td>
</tr>
<tr>
<td>Local</td>
<td></td>
</tr>
<tr>
<td>Pain</td>
<td>77</td>
</tr>
<tr>
<td>Warmth</td>
<td>54</td>
</tr>
<tr>
<td>Erythema</td>
<td>29</td>
</tr>
<tr>
<td>Induration</td>
<td>11</td>
</tr>
<tr>
<td>Systemic</td>
<td></td>
</tr>
<tr>
<td>Chills</td>
<td>9</td>
</tr>
<tr>
<td>Nausea</td>
<td>23</td>
</tr>
<tr>
<td>Myalgie</td>
<td>49</td>
</tr>
<tr>
<td>Rash</td>
<td>40</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>14</td>
</tr>
<tr>
<td>Headache</td>
<td>34</td>
</tr>
<tr>
<td>Temperature ≥38°C</td>
<td>6</td>
</tr>
<tr>
<td>Other</td>
<td></td>
</tr>
<tr>
<td>Stayed home</td>
<td>14</td>
</tr>
<tr>
<td>Analgesic or antipyretic</td>
<td>23</td>
</tr>
</tbody>
</table>

**NOTE.** Data are percentage of subjects. Reactions are summarized as the worst severity following any injection.

dose (figure 2). Combining the SF2 gp120 BAb titers from different doses of CM 235 over each SF2 dose, regardless of the dosage of subtype E vaccination, the GMTs of the BAb were 281, 1623, and 2337 for the doses of SF2 at 0, 25, and 50 µg, respectively. Anti-CM235 gp120 BAb titers in recipients of bivalent (E plus B gp120) and monovalent (E only) groups were similar (figure 1). There was no evidence of antigenic interference. Of interest, immunization with CM235 gp120 (E) alone resulted in BAb that cross-reacted with SF2 gp120 (B) antigen, although the response was augmented by >5-fold when SF2 gp120 was included in the immunization regimen.

**NAb response.** Previous work has shown that the vaccination of humans with TCLA HIV-1 gp120 results in the generation of NAb against homologous TCLA HIV strains and some additional neutralization of other strains within the same subtype [22]. We first screened for NAb to the heterologous TCLA subtype E strain NP03 (X4) and the homologous TCLA subtype B strain SF2 (X4). Pre- and postvaccination serum samples from 287 vaccine recipients and 80 placebo recipients were studied for neutralization of NP03 and SF2. Serum samples from 242 (84%) of these vaccine recipients neutralized NP03; 235 (82%) samples neutralized SF2. Two of the 80 placebo recipients (2.5%) had ≥50% neutralization of NP03, whereas 5 (6%) had NAb against SF2. There were no statistically
Figure 2. Geometric mean titer of anti-gp120 SF2 after vaccination with various doses (in micrograms) of subtype B and subtype E vaccines. Titers \( \geq 100 \) were considered to be positive; nonpositive serum samples were assigned a titer of 50.

Table 2. Neutralizing antibody titers to NP03/H9 in the subgroup of vaccine recipients who received the highest dose of gp120 SF2 ( ), by dose of Thai E (CM235) gp120 vaccine received.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosage of gp120, mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CM235</td>
<td>25</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>SF2</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Neutralization titer, geometric mean(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% neutralization</td>
<td>147</td>
<td>169</td>
<td>155</td>
</tr>
<tr>
<td>90% neutralization</td>
<td>1</td>
<td>5</td>
<td>12</td>
</tr>
</tbody>
</table>

**NOTE.** A reciprocal serum dilution of \( \geq 8 \) was considered to be positive.

\(^a\) No statistically significant differences between dose groups.
The early identification and extensive characterization of the HIV epidemic in Thailand have allowed for the testing of several HIV vaccine candidates matched to viral subtypes (E and B) currently circulating in that country. The TAVEG has reported that a subtype B, TCLA gp120 SF2 vaccine (also using the MF59 adjuvant) in Thai adults was safe and induced cross-clade CD4+ lymphoproliferative responses, as well as homologous TCLA NAb [20]. A gp120 subtype B TCLA MN vaccine (with alum adjuvant) showed a similar profile in a phase 1 trial in Thailand [21]. The current trial used a coformulation of the previous SF2 gp120 vaccine with a gp120 derived from the subtype E, R5-tropic primary isolate CM235. This report on the safety and immunogenicity of gp120 CM235 alone and in combination with SF2 gp120 is, to our knowledge, the first description of the outcome of vaccination with an R5 gp120 candidate vaccine in humans.

Similar to the safety profile of other gp120 and gp160 subunit vaccines [31, 32], gp120 CM235 alone or combined with SF2 gp120 (with MF59 adjuvant) was well tolerated. The reported reactogenicity was similar to that of SF2 gp120/MF59 alone in Thai adults [20] and was comparable to that in the control group using adjuvant alone. There were no vaccine-related serious adverse events, and no immunizations had to be withheld because of intolerability.

Clinical trials of HIV-1 TCLA (X4-using) envelope gp120 and gp160 subunit products (e.g., SF2, IIIB, and MN) have shown the induction of high-titer, antigen-specific BAb [4, 10–19]. In the present trial, the magnitude of the CM235 gp120-induced BAb response was independent of the presence of SF2 gp120 antigen, suggesting that there was no antigenic interference or augmentation between the CM235 and SF2 gp120s. Similar results were obtained using the bivalent gp120 A244/MN antigens (AIDSVAX B/E) (P.P., P. W. Berman, B. Phonrat, P. Suntharasamai, S. Raktham, L. Srisuwanvilai, K. Hirunras, D. Kityaporn, J. Kaewkangwal, S. Migasena, H. W. Sheppard, E. Li, M. Chernow, M. L. Peterson, R. Shibata, W. L. Heyward, and D. P. Francis, unpublished data). The level of anti-CM235 gp120 BAb was independent of the dose of CM235 gp120 received. In 95% of vaccine recipients, 2 doses of vaccine were required before antibody could be detected. For all immunization regimens, the highest BAb titer for both antigens was observed after the third dose. After vaccination with gp120 CM235 only, there was induction of a low level of BAb that cross-recognized subtype B gp120. It is not known whether the induction of cross-reactive antibody is a general feature of immunization with R5 tropic (vs X4) gp120 or a particular feature of immunization with gp120 CM235.

The SF2 and MN gp120 vaccines tested previously in Thailand induced both BAbs and NAb against homologous SF2 and MN in T cell lines [20, 21]. The vaccination of baboons with CM235 gp120 resulted in the induction of antibody that was capable of neutralizing a number of X4 subtype E viruses [27], but no neutralization of the homologous primary isolate CM235 was found. In the present study, NABs against TCLA HIV NP03 (subtype E) and SF2 (subtype B) were found in >80% of vaccine recipients. Analogous to previous reports of

### DISCUSSION

The early identification and extensive characterization of the HIV epidemic in Thailand have allowed for the testing of several HIV vaccine candidates matched to viral subtypes (E and B) currently circulating in that country. The TAVEG has reported that a subtype B, TCLA gp120 SF2 vaccine (also using the MF59 adjuvant) in Thai adults was safe and induced cross-clade CD4+ lymphoproliferative responses, as well as homologous TCLA NAb [20]. A gp120 subtype B TCLA MN vaccine (with alum adjuvant) showed a similar profile in a phase 1 trial in Thailand [21]. The current trial used a coformulation of the previous SF2 gp120 vaccine with a gp120 derived from the subtype E, R5-tropic primary isolate CM235. This report on the safety and immunogenicity of gp120 CM235 alone and in combination with SF2 gp120 is, to our knowledge, the first description of the outcome of vaccination with an R5 gp120 candidate vaccine in humans.

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### Table 3. Lymphocyte stimulation indices (LSIs) of reduced carboxy-methylated gp120 SF2 and CM235 vaccines at baseline (Visit 1) and after the third vaccination (Visit 6) of the subgroup of vaccine recipients who received the high dose of SF2, by the dose of CM235 gp120 received.

<table>
<thead>
<tr>
<th>Dose of CM235 vaccine, visit</th>
<th>Vaccine</th>
<th>SF2</th>
<th>CM235</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 μg</td>
<td>1</td>
<td>1 (1–7)</td>
<td>1 (1–6)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>44 (1–193)</td>
<td>21 (1–107)</td>
</tr>
<tr>
<td>50 μg</td>
<td>1</td>
<td>1 (1–9)</td>
<td>2 (1–15)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>40 (1–242)</td>
<td>11 (1–108)</td>
</tr>
<tr>
<td>100 μg</td>
<td>1</td>
<td>1 (1–14)</td>
<td>1 (1–5)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>25 (1–148)</td>
<td>11 (1–86)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are geometric mean LSI (range). The maximum response among the 3 antigen dosages used for LPA stimulation was chosen as the representative LSI.

V.P., R. McLinden, A. Sambor, A.E.B., B. Phonrat, K. Rungrueng-thanakit, A.-M.D., M. L. Robb, J.G.M., D.L.B., and the TAVEG, unpublished data; that there was a correlation between CM235 gp120 BAb and the neutralization of CM235M4/C4.6, but not NP03 or CM235, in PBMCs.

**Lymphoproliferation responses.** The lymphoproliferative responses to PHA and tetanus toxoid were equivalent in magnitude and frequency at both time points assayed (data not shown), which demonstrates that the PBMCs were well cryopreserved and retained their functional ability to respond to both mitogen and recall antigen. The percentage of responders to rcm gp120 SF2 in each of the 3 Thai E dose groups was >95% after 3 immunizations, whereas the percentage of responders to rcm gp120 CM235 was the highest (97%) for the combination with the lowest dose of Thai E antigen (25 μg) and lower with the higher dose of Thai E antigen (75% for 50 μg and 78% for 100 μg of CM235). The variability of the responses, as measured by the LSI geometric means, showed a greater range for the rcm gp120 SF2, compared with gp120 CM235 (table 3). The percentage of responders to different concentrations of envelope proteins at day 0 (baseline) was 3%–6% for rcm gp120 SF2 and 6%–16% to rcm gp120 CM235.
NAb from TCLA (X4) gp120, no significant neutralization of the vaccine-strain primary isolate CM235 was found in PBMCs. However, antibody capable of neutralizing the homologous, adapted CM235M4/C4.6 virus was present in 56% of vaccine recipients. The mechanism of increased sensitivity of the CM235M4/C4.6 virus to vaccine-induced NAb is not understood (J.K., P.P., C.K., T.C., J. Mascola, S. Frankel, M.d.s., V.P., R. McLinden, A. Sambor, A.E.B., B. Phonrat, K. Rungruangthanakit, A.-M.D., M. L. Robb, J.G.M., D.L.B., and the TAVEG, unpublished data); it is not mediated by an increase in V3-positive charge.

The subset of volunteers studied (those who received 50 μg of gp120 SF2 in combination with different doses of gp120 CM235) mounted good T helper responses to both immunogens, comparable to the previous phase 1/2 trial with gp120 SF2 alone [20]. Although there was a higher background for the Thai E immunogen, compared with the gp120 SF2, the group that received the lowest dose of gp120 CM235 had the best lymphoproliferative response to both immunogens. There was no evidence that gp120 CM235 adversely affected the proliferative response to gp120 SF2.

Taken together, these data demonstrate that the bivalent combination of gp120 CM235 and SF2 is safe, well-tolerated, and immunogenic in low-risk, HIV-seronegative Thai adults. Further human studies of the combined vaccines, after priming with canarypox-vectored HIV vaccine containing subtype B and E antigens, should be pursued in Thailand. It may provide further information on potential antibody specificity, both breadth and magnitude, that would be useful in the context of the planned prime-boost phase 3 trial.

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Acknowledgments

We thank the nursing staffs of the Thai AIDS Evaluation Group for their dedicated study implementation, and Prasong Srisaenchai, Somchintana Chanthong, and Wisut Lokpichart for assistance in data management and analysis.

References