Safety, Immunogenicity, and Surrogate Markers of Clinical Efficacy for Modified Vaccinia Ankara as a Smallpox Vaccine in HIV-Infected Subjects

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Background. Human immunodeficiency virus (HIV)–infected persons are at higher risk for serious complications associated with traditional smallpox vaccines. Alternative smallpox vaccines with an improved safety profile would address this unmet medical need.

Methods. The safety and immunogenicity of modified vaccinia Ankara (MVA) was assessed in 91 HIV-infected adult subjects (CD4+ T-cell counts, \( \geq 350 \) cells/mm\(^3\)) and 60 uninfected volunteers. The primary objectives were to evaluate the safety of MVA and immunogenicity in HIV-infected and uninfected subjects. As a measure of the potential efficacy of MVA, the ability to boost the memory response in people previously vaccinated against smallpox was evaluated by the inclusion of vaccinia-experienced HIV-infected and HIV-uninfected subjects.

Results. MVA was well tolerated and immunogenic in all subjects. Antibody responses were comparable between uninfected and HIV-infected populations, with only 1 significantly lower total antibody titer at 2 weeks after the second vaccination, while no significant differences were observed for neutralizing antibodies. MVA rapidly boosted the antibody responses in vaccinia-experienced subjects, supporting the efficacy of MVA against variola.

Conclusions. MVA is a promising candidate as a safer smallpox vaccine, even for immunocompromised individuals, a group for whom current smallpox vaccines have an unacceptable safety profile.

Clinical Trials Registration. NCT00189904.

Keywords. Smallpox; vaccine; HIV; MVA.

The World Health Organization endorsed the global eradication of smallpox in 1980 [1]. Nevertheless, serious concerns about the reemergence of variola virus (VARV) as a global pathogen persist, which has led to the resumption of immunizations with traditional smallpox vaccines based on replicating vaccinia viruses (VACV) for military personnel and so-called first responders [2]. While traditional smallpox vaccines have proven to be highly protective, they have the potential to cause serious complications, including progressive vaccinia, eczema vaccinatum, generalized vaccinia, encephalitis, and myopericarditis [3–5]. People with immunodeficiency and chronic skin conditions are especially at risk to develop these vaccine complications, and therefore the use of traditional smallpox vaccines is contraindicated for people infected with human immunodeficiency virus (HIV) or...
with a diagnosis of atopic dermatitis [2]. Notably, dissemni-
ted vaccinia following smallpox vaccination has been reported
in a US military recruit with asymptomatic HIV infection and
a normal CD4+ T-cell count [6], and the inadvertent inocula-
tion of contacts by vaccinees having received traditional small-
pox vaccines poses an additional risk for subjects with
impaired immune function [7, 8]. Such examples outline the
unmet medical need for a safer smallpox vaccine for popula-
tions at risk of experiencing complications from traditional
smallpox vaccines.

Historically, several attempts were made during the eradica-
tion of VARV to attenuate the smallpox vaccine. One success-
ful attempt was the poxvirus-modified vaccinia Ankara
(MVA) that was used during the 1970s in >120 000 people,
including children with immunodeficiencies, for priming prior
to administration of a traditional smallpox vaccine [9, 10].
MVA was derived by >570 passages of the parental chorioal-
lantoic vaccinia Ankara virus in primary chicken embryo
fibroblast cells; MVA became replication restricted to avian cells
[11]. The attenuated properties have subsequently led numer-
ous groups to investigate the potential of MVA as a safe
vaccine vector [12], in addition to a standalone smallpox
vaccine. To date, studies have shown the vaccine to be well
tolerated in healthy adults and to induce immune responses
comparable to those of traditional smallpox vaccines [13–15].
These clinical data are supported by findings in a variety of
animal models that MVA induces efficacy that is comparable
[16, 17] or even superior [18, 19] to that of traditional smallpox
vaccines. Promisingly, vaccinations with MVA have been shown
to be as effective as a traditional smallpox vaccine in eliciting
VARV-neutralizing immune responses in healthy adults [20].

Because it is no longer possible or ethical to demonstrate
efficacy against VARV in a clinical setting, new smallpox vac-
cines such as MVA that are given intramuscularly and subcu-
taneously must rely on acceptable correlates of protection to
demonstrate efficacy. As a consequence, the recent approval of
the replicating smallpox vaccine ACAM2000, a New York City
Board of Health VACV strain, was based in part on the ability
of this vaccine to boost the immune response in people previ-
ously vaccinated against smallpox. The ability to boost the ex-
isting B-cell memory is considered more important in
demonstrating efficacy in this vaccinia-experienced population
than the vaccine take [21]. Such immunological correlates will
be important in the demonstration of the efficacy of MVA
against VARV.

To evaluate the effectiveness of MVA as a smallpox vaccine
in a population with higher risk of complications associated
with traditional smallpox vaccines, we performed a phase I/II
clinical study comparing the safety and immunogenicity of
MVA in vaccinia-naive HIV-infected subjects (CD4+ T-cell
counts, >350/µL) and uninfected subjects. To evaluate
whether MVA was capable of an anamnestic response,
vaccinia-experienced HIV-infected and uninfected subjects
were enrolled as 2 additional groups.

METHODS

Vaccine
The MVA strain used in the current study was derived from
the MVA vaccine licensed in Germany [10, 11] by additional
passages and serial dilutions in chicken embryo fibroblast cells
and has been shown not to replicate in human cells or severely
immunocompromised animals [22]. MVA (Imvanune) was
produced by IDT Biologika (Dessau-Roßlau, Germany) ac-
cording to good manufacturing practice and was provided by
Bavarian Nordic A/S (Kvistgaard, Denmark) as a refrigerated
freeze-dried product with a nominal titer of 1 × 108 median
tissue culture infective doses. The vaccine was reconstituted
in 0.5 mL sterile water prior to injection.

Participants and Vaccination Schedule
After approval by the relevant institutional review boards, the
trial was initiated at 5 US centers. All study related procedures
followed the Declaration of Helsinki principles, the ICH-GCP
requirements, and the US Code of Federal Regulation appli-
cable for clinical trials, and all volunteers provided a written
informed consent. Subjects eligible for enrollment were men
(age, 18–49 years) or nonpregnant women (age, 18–55 years)
either with (vaccinia experienced) or without (vaccinia naive)
previous smallpox vaccination. HIV-infected participants had
to be receiving stable or no highly active antiretroviral therapy
(HAART) for >6 months prior to enrollment, with a plasma
HIV-1 RNA load of <400 copies/mL and a CD4+ T-cell count
of ≥350 cells/mm3 determined from 2 measurements at least
4 weeks apart; satisfaction of these requirements was also nec-
essary for all vaccinia-naive HIV-infected participants before
they received their second vaccination. All uninfected subjects
tested HIV seronegative by enzyme-linked immunosorbent
assay (ELISA). Subjects were required to undergo renal,
hepatic, and hematology studies and electrocardiography
(ECG) and have no clinically significant findings. Exclusion
criteria included (1) a ≥10% risk of developing a myocardial
infarction or coronary death within 10 years, using the Na-
tional Cholesterol Education Program's risk assessment tool;
(2) an immediate family member with onset of ischemic heart
disease before age 50 years; and (3) a history of active autoim-
mune disease, diabetes, malignancy, organ transplantation,
or clinically significant and severe illness.

All enrolled subjects received a subcutaneous MVA vac-
jection in the nondominant upper arm at visit 1 (day 0). Vaccinia-
naive subjects received a second vaccination with the same
dose 4 weeks later. All subjects had follow-up visits 2 weeks
after each vaccination, at week 8, and at least 26 weeks after
the last study vaccination.
Safety Assessments
Occurrence, relationship, and intensity of any unsolicited adverse event (AE), either spontaneously reported by the subject at any time or detected during the follow-up visits by the investigator or by safety laboratory tests, was recorded at each study visit within a 29-day period after vaccination. Cardiac safety was evaluated at baseline and at 2 weeks after each vaccination, including ECG and measurement of cardiac enzyme levels. An additional measurement of cardiac enzyme levels was made 4 weeks after the last vaccination. Solicited AEs constituted predefined, expected local reactions (eg, erythema, swelling, induration, and pain) and general symptoms (pyrexia, headache, myalgia, chills, nausea, or fatigue) and were assessed for occurrence, intensity, and duration by the subjects in a diary card that they kept for 8 days following each vaccination. The intensity of unsolicited and solicited AEs was analyzed according to predefined grades, with grade 3 defining severe AEs. Grade 3 local injection site reactions of erythema, swelling, and induration were defined as reactions with a diameter of ≥100 mm. Grade 3 pain was pain that prevented normal activity; the severity of general symptoms was considered grade 3 if daily activity was prevented. Headache or myalgia that was “disabling” and fatigue resulting in being “bedridden” were rated a grade 4 (ie, potentially life threatening). Grade 3 pyrexia was defined as a temperature of ≥39.0 to <40.0°C; grade 4 was defined as a temperature of ≥40.0°C.

Immunogenicity
Antibody (immunoglobulin G) responses to MVA were measured using an automated ELISA as previously described [15]. The plaque reduction neutralization test (PRNT) was performed by FOCUS Diagnostics (Cypress, CA) as previously described [15] but with a VACV Western Reserve strain as the test virus. Antibody titers below the assay cutoff of 10 were assigned an arbitrary value of 1 for the purpose of calculations, whereas a PRNT value of ≥10 was considered seropositive. Geometric mean titer (GMT) was calculated using the antilogarithm of the mean of the log_{10} titer transformation. Seroconversion by PRNT was defined as seropositivity, for individuals who were seronegative prior to vaccination, or as an antibody titer that increased ≥2-fold from baseline, for subjects with preexisting antibody titers.

Statistical Analysis
Statistical analyses were performed using SAS software on 2 data sets. The sample size calculations were based on the incidence of serious AEs, to provide a probability of 95% to detect a serious AE with an incidence of at least 10% (in a sample size of 30) or 5% (in a sample size of 60). Also, a sample size of 30 in each group would have 80% power to detect a difference in means of 15% in the ELISA results, using a 2-group t test with a 5% 2-sided significance level. The full-analysis set included all individuals who received at least 1 vaccination and was used to analyze safety. The per-protocol analysis set included all subjects without major protocol violations and was used to assess the immunogenicity. To compare the HIV-infected group with the uninfected group, a Wilcoxon test was used. Additional immunogenicity analyses were performed using a variety of tests, as indicated in Results.

RESULTS

Participants and Demographic Data
A total of 151 individuals received at least 1 MVA vaccination and were included in the full analysis set (Figure 1). Sixteen subjects were excluded from the per-protocol set (n = 135) because they had no baseline anti-vaccinia ELISA (in 8 cases), missed visits (in 2), had visits out of the study window (in 1), did not receive all vaccinations (in 1), had reactive hepatitis C virus antibodies (in 1), were participating in another research trial (in 1), received a hepatitis vaccination on the day of a study vaccination (in 1), and had clinically significant abnormal results of liver function tests at screening (in 1).

On average, the vaccinia-experienced individuals (HIV-infected and uninfected) were >10 years older than the vaccinia-naive individuals (Table 1). More men than women were recruited into the HIV-infected groups, while the reverse was true for the uninfected controls. The majority of recruited subjects were white. However, more African Americans were included in the HIV-infected groups than in the uninfected groups. HAART was being used by 97% of HIV-infected subjects (3 HIV-infected subjects were not receiving HAART).

Safety
No clinically meaningful changes in ECG findings, hematology and biochemistry values, or vital signs were observed for any subject, and no significant differences between the study groups were recorded. Mean CD4+ T cell counts did not significantly change from baseline to postvaccination visits in the HIV-infected subjects (Table 1). The most common unsolicited AE was injection site pruritus following vaccination(s) with MVA (Table 2). While 5 unlikely related AEs (pharyngolaryngeal pain, dizziness, headache, hypertension, and anxiety) in 1 vaccinia-naive uninfected subject prevented the administration of the second vaccination, no vaccine-related AE led to study withdrawal.

Most subjects reported transient mild-to-moderate reactions (ie, grade 1 or 2) at the injection site following vaccination(s) with MVA (Table 3). Grade ≥ 3 solicited local AE were infrequent (≤7%) and not significantly different between HIV-infected and uninfected subjects. Pain at the vaccination site was significantly more frequent than the other solicited local AEs. However, aside from pain at the injection site, vaccination(s) with MVA appeared to be better tolerated in HIV-infected
subjects than in uninfected individuals. There was a significantly lower incidence of erythema, swelling, induration, and injection site pruritus in the HIV-infected groups (vaccinia-naive and vaccinia-experienced combined; P < .05). Differences in local AEs did not reach statistical significance between vaccinia-naive and vaccinia-experienced HIV-infected subjects. No other significant differences were observed between the vaccinia-naive and vaccinia-experienced groups. For general solicited AEs, the highest incidence was reported for headache and myalgia.

During the active phase of the study (ie, up to and including the day of the last vaccination), 3 unrelated serious AEs (foot fracture, noncardiac chest pain related to a respiratory viral syndrome, and elevated platelet count associated with chronic leukemia) were reported in uninfected subjects. Two additional serious AEs (left hip infection after arthroplasty and cardiomyopathy with congestive heart failure) were reported during the 26-week follow-up phase of the study. The latter case was reported for a vaccinia-naive HIV-infected woman 133 days after the last MVA vaccination. This patient also had several concomitant medical conditions, including dyspnea, pleural effusion, hypertension, obesity, glaucoma, and osteopenia, as well as a history of heart surgery during childhood. She was hospitalized for 10 days and later released in a stable condition with cardiac medications. Unknown to the site staff, this subject had been concomitantly participating in a growth hormone releasing hormone (GH-RH) trial for...
treatment of lipodystrophy, and the event of “congestive cardiac failure” had been recorded as being possibly related to GH–RH in this trial. The independent data safety monitoring board overseeing the MVA development program concluded that this specific serious AE did not represent an increased risk to subjects for developing cardiac events.

### Table 1. Demographic Data and Human Immunodeficiency Virus (HIV) Status in the Full Analysis Set of 151 Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HIV Infected, Vaccinia Naive (n = 30)</th>
<th>HIV Infected, Vaccinia Experienced (n = 61)</th>
<th>Uninfected, Vaccinia Naive (n = 30)</th>
<th>Uninfected, Vaccinia Experienced (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>31.4 ± 5.6</td>
<td>43.1 ± 4.4</td>
<td>26.8 ± 5.8</td>
<td>44.9 ± 5.5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>174 ± 11</td>
<td>171 ± 11</td>
<td>169 ± 8</td>
<td>168 ± 10</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>88 ± 25</td>
<td>81 ± 16</td>
<td>81 ± 16</td>
<td>87 ± 28</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22 (73.3)</td>
<td>42 (68.8)</td>
<td>8 (26.7)</td>
<td>9 (30.0)</td>
</tr>
<tr>
<td>Female</td>
<td>8 (26.7)</td>
<td>19 (31.1)</td>
<td>22 (73.3)</td>
<td>21 (70.0)</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>17 (56.7)</td>
<td>36 (59.0)</td>
<td>26 (86.7)</td>
<td>22 (73.3)</td>
</tr>
<tr>
<td>African American</td>
<td>12 (40.0)</td>
<td>25 (41.0)</td>
<td>2 (6.7)</td>
<td>6 (20.0)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td>Oriental/Asian</td>
<td>0</td>
<td>0</td>
<td>1 (3.3)</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>1 (3.3)</td>
<td>1 (3.3)</td>
<td>1 (3.3)</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td>CD4+ T-cell count, cells/mm³</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>726 ± 233</td>
<td>743 ± 287</td>
<td>NA</td>
<td>709 ± 262</td>
</tr>
<tr>
<td>End of trial</td>
<td>775 ± 386</td>
<td>709 ± 262</td>
<td>NA</td>
<td>59 (97)</td>
</tr>
<tr>
<td>HAART</td>
<td>29 (97)</td>
<td>NA</td>
<td>59 (97)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Data are means ± SD or no. (%) of subjects.
Abbreviations: HAART, highly active antiretroviral therapy; NA, not applicable.

### Table 2. Most Common Unsolicited Adverse Events (AEs) Among 151 Subjects in the Full Analysis Set During the 28-Day Follow-up Period After Both Vaccinations

<table>
<thead>
<tr>
<th>Location, AE, Grade</th>
<th>HIV Infected, Vaccinia Naive (n = 30)</th>
<th>HIV Infected, Vaccinia Experienced (n = 61)</th>
<th>Uninfected, Vaccinia Naive (n = 30)</th>
<th>Uninfected, Vaccinia Experienced (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection site</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pruritus</td>
<td>4 (13)</td>
<td>6 (10)</td>
<td>9 (30)</td>
<td>11 (37)</td>
</tr>
<tr>
<td></td>
<td>≥3</td>
<td>0</td>
<td>0</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Bruising</td>
<td>0</td>
<td>3 (10)</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>≥3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Warmth</td>
<td>0</td>
<td>2 (7)</td>
<td>2</td>
<td>1 (2)</td>
</tr>
<tr>
<td></td>
<td>≥3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nervous system</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dizziness</td>
<td>0</td>
<td>2 (7)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>≥3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Data are for AEs in which >1 event was reported. A subject may have AEs reported in >1 category.
Abbreviations: HIV, human immunodeficiency virus; vacc, vaccinations.
Immunogenicity

As illustrated in Figure 2, the kinetics of the total and neutralizing antibody responses induced following the vaccinations with MVA in the vaccinia-naive subjects were comparable, irrespective of the subjects’ HIV status. In the majority of the vaccinia-naive subjects, total antibody responses were already detected 2 weeks after the primary vaccination, with 83% and 78% seroconversion in the HIV-infected and uninfected individuals, respectively (Figure 2A). The magnitude of the response was comparable between the 2 vaccinia-naive groups at this early time point, with GMTs of 88 and 98 in the HIV-infected and uninfected subjects. The GMT further increased to 225 in the uninfected subjects 4 weeks after the primary vaccination, while the titers remained stable in the HIV-infected subjects (Figure 2B). The peak total antibody response was recorded on day 42, 2 weeks after the second vaccination, with

Table 3. Overall Incidence of Local and General Solicited Adverse Events (AEs), by Maximum Intensity During the 8-Day Follow-up Period After 2 (Vaccinia-Naive Subjects) or 1 (Vaccinia-Experienced Subjects) Vaccination, Among 151 Subjects in the Full Analysis Set

<table>
<thead>
<tr>
<th>AE, Grade(a)</th>
<th>HIV Infected, Vaccinia Naive (n = 30)</th>
<th>Uninfected, Vaccinia Naive (n = 30)</th>
<th>HIV Infected, Vaccinia Experienced (n = 61)</th>
<th>Uninfected, Vaccinia Experienced (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, No. (%)</td>
<td>Subjects, No. (%)</td>
<td>Subjects, No. (%)</td>
<td>Subjects, No. (%)</td>
<td>Subjects, No. (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>26 (87)</td>
<td>29 (97)</td>
<td>49 (80)</td>
<td>28 (93)</td>
</tr>
<tr>
<td>≥3</td>
<td>0</td>
<td>2 (7)</td>
<td>2 (3)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Erythema</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>17 (57)</td>
<td>24 (80)</td>
<td>27 (44)</td>
<td>23 (77)</td>
</tr>
<tr>
<td>≥3</td>
<td>0</td>
<td>0</td>
<td>1 (2)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Swelling</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>17 (57)</td>
<td>19 (63)</td>
<td>22 (36)</td>
<td>19 (63)</td>
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<td>≥3</td>
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<td>0</td>
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<td>Induration</td>
<td></td>
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<tr>
<td>Any</td>
<td>12 (40)</td>
<td>17 (57)</td>
<td>18 (30)</td>
<td>17 (57)</td>
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<tr>
<td>≥3</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>General</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature increase</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Any</td>
<td>2 (7)</td>
<td>5 (17)</td>
<td>6 (10)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>≥3</td>
<td>0</td>
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<td>1 (2)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Headache</td>
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</tr>
<tr>
<td>Any</td>
<td>12 (40)</td>
<td>15 (50)</td>
<td>20 (33)</td>
<td>14 (47)</td>
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<td>1 (3)</td>
<td>2 (7)</td>
<td>1 (2)</td>
<td>1 (3)</td>
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<td>Myalgia</td>
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</tr>
<tr>
<td>Any</td>
<td>14 (47)</td>
<td>15 (50)</td>
<td>25 (41)</td>
<td>14 (47)</td>
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<td>1 (3)</td>
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<td>Chills</td>
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<td>Any</td>
<td>9 (30)</td>
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<td>8 (13)</td>
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<tr>
<td>≥3</td>
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<td>2 (7)</td>
<td>1 (2)</td>
<td>1 (3)</td>
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<td>Nausea</td>
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<tr>
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<td>7 (23)</td>
<td>8 (27)</td>
<td>15 (25)</td>
<td>10 (33)</td>
</tr>
<tr>
<td>≥3</td>
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<td>0</td>
<td>1 (2)</td>
<td>2 (7)</td>
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<td>Fatigue</td>
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<tr>
<td>Any</td>
<td>13 (43)</td>
<td>12 (40)</td>
<td>24 (39)</td>
<td>10 (33)</td>
</tr>
<tr>
<td>≥3</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td>4 (7)</td>
<td>0</td>
</tr>
</tbody>
</table>

A subject may have findings in >1 category.
Abbreviation: HIV, human immunodeficiency virus.

*Intensity grade ≥3 definitions: pain, spontaneously painful or preventing normal daily activities; erythema, swelling, or induration, largest surface diameter of ≥100 mm; temperature increase, ≥39°C; headache, myalgia, nausea, or fatigue, preventing normal daily activities.
a GMT of 778 and 1939 in the HIV-infected and uninfected subjects, respectively. This was the only time point with a significant difference \((P = .01)\) in total antibody titers between the 2 groups.

Within the vaccinia-naive population, there were no significant differences in the PRNT titers at any time point between the 2 groups. The highest neutralizing antibody response was measured 2 weeks after the second vaccination in the uninfected subjects, with 96% seroconversion (Figure 2C) and a peak PRNT GMT of 177 (Figure 2D). This was not significantly different from the 89% seroconversion and GMT of 95 presented at the same time point by the HIV-infected subjects.

The majority (74%–89%) of the vaccinia-experienced subjects, irrespective of their HIV status, had antibodies detected, albeit at low levels, by both ELISA and PRNT prior to vaccination, with GMTs of 18–69, confirming their status as previously vaccinated against smallpox. In the vaccinia-experienced subjects, the kinetics of the antibody response was characteristic of a booster vaccination, with the highest total and neutralizing antibody responses detected 2 weeks after the single vaccination with MVA (Figure 2A and 2C). The kinetics in terms of the antibody magnitude and timing were comparable between HIV-infected and uninfected subjects, as measured by ELISA and PRNT (Figure 2B and 2D). Two weeks after the booster vaccination with MVA there were a 16- and 17-fold increase in the total antibody titer, with ELISA seroconversion rates of 92% and 96% and a peak GMT of 684 and 1176 (infected and uninfected, respectively; Figure 2A and 2B). Differences observed in the neutralizing titers induced by MVA between the vaccinia-experienced HIV-infected and uninfected subjects were not significant. Peak neutralizing seroconversion rates were 91% and 93% and GMTs 336 and 761 in the HIV-infected and uninfected subjects, respectively (Figure 2C and 2D).
Six months after the last vaccination, there was a decline in the titers detected by both ELISA and PRNT in all study groups. However, most subjects in all groups still had detectable ELISA and PRNT titers. The rates of the decline in the antibody response were comparable between the various groups. In all groups the antibody titers and seroconversion rates measured by PRNT were lower than the total antibody responses measured by ELISA. In all 4 study groups, a significant correlation between ELISA and PRNT could be shown for all study visits, with correlation coefficients ranging from 0.53 to 0.79 (P < .005 for all).

**Comparison of Antibody Responses in Vaccinia-Naive and Vaccinia-Experienced Subjects**

A single vaccination with MVA boosted both the total antibody and neutralizing antibody responses in the vaccinia-experienced subjects. Peak total antibody responses in the uninfected vaccinia-naive subjects were either not significantly different or superior to those in the HIV-infected and uninfected vaccinia-experienced populations, respectively (Table 4). Both vaccinia-experienced populations had higher peak neutralizing antibody titers, compared with the respective vaccinia-naive groups, regardless of their HIV status.

**DISCUSSION**

While there have been studies investigating recombinant MVA-based HIV vaccines [23–25], this trial represents the first clinical investigation of safety and immunogenicity for MVA as a smallpox vaccine in HIV-infected subjects, a population for which the current licensed vaccines based on a fully replicating VACV are contraindicated [2]. MVA was shown to be safe and well tolerated in the uninfected volunteers, regardless of their previous smallpox vaccination status, confirming the findings from earlier clinical trials [13–15]. Importantly, the safety profile of MVA in the HIV-infected subjects was comparable, if not better, in terms of the local reactions than that in the uninfected subjects. Only 7% of subjects in the trial had any grade 3 adverse event after vaccination. The low number of serious AEs recorded in the trial supports the outstanding safety profile of MVA to date. A single serious AE of cardiomyopathy and congestive heart failure assessed as possibly related to the study vaccine by the study investigator was not considered by the independent data safety monitoring board to be due to MVA. The reasons for the high rates of myopericarditis following vaccinations with traditional smallpox vaccines are not entirely clear but seem to be associated with an inflammatory response related to the replicating nature of VACV [26–28]. Whether HIV-infected subjects are more prone to the induction of myopericarditis by VACV is unknown, but besides the serious AE mentioned above, no findings in terms of cardiac symptoms or clinically abnormal ECGs were recorded in the current study, supporting the observations made in an earlier phase I trial in healthy subjects [29]. Indeed, no cases of myopericarditis have been recorded in any of the studies performed using MVA as a smallpox vaccine, despite the close monitoring of cardiac events [30].

The total and neutralizing antibody titers induced by MVA in the vaccinia-naive and experienced subjects were mostly lower in HIV-infected groups, differences that were generally not statistically significant owing to the sample size. In addition, they were similar in kinetics and magnitude to those...
observed in earlier MVA trials and comparable to those stimulated by a traditional vaccine with proven protection against smallpox [13–15]. In a recent study comparing MVA to Dryvax in healthy vaccinia-naive adults, the GMT for total antibodies (using the same validated assay) was 215 for subjects (n = 13) receiving Dryvax, which is at least a 3-fold lower level than the responses recorded in the HIV-infected subjects who received MVA in this trial [14].

The ability of MVA to rapidly boost the antibody responses in all vaccinia-experienced subjects, regardless of their HIV status, supports the observation that traditional smallpox vaccines induced a long-lived B-cell memory [31, 32]. Historically, booster vaccinations with traditional smallpox vaccines provided a robust protection [33], although the vaccine take, which is an accepted marker of efficacy against smallpox, was often attenuated in these vaccinia-experienced subjects. Indeed, recently the ability to boost the antibody response in vaccinia-experienced subjects has been suggested to be more important than the take rate following revaccination with a traditional smallpox vaccine in this population [21]. Therefore, the anamnestic response recorded following a booster vaccination with MVA is strong evidence to suggest the efficacy of this vaccine in these vaccinia-experienced subjects.

Interestingly, these presumably protective total antibody responses measured in the vaccinia-experienced populations were shown to be either not statistically different from or superior to the peak response observed in the vaccinia-experienced populations. These data are encouraging as a potential surrogate marker of efficacy for MVA in vaccinia-naive subjects. The neutralizing titers were, however, higher in the vaccinia-experienced subjects, irrespective of their HIV status, presumably because of the induction of higher-affinity antibodies following the repeat exposure to the live virus. Indeed, traditional smallpox vaccines also induce a 2-fold higher neutralizing titer following the repeat exposure to the live virus. Indeed, recently the ability to boost the antibody response in vaccinia-experienced subjects has been suggested to be more important than the take rate following revaccination with a traditional smallpox vaccine in this population [21]. Therefore, the anamnestic response recorded following a booster vaccination with MVA is strong evidence to suggest the efficacy of this vaccine in these vaccinia-experienced subjects.

Overall, the results of this clinical trial confirm the favorable safety profile of MVA as a smallpox vaccine, which was shown to be equally safe and well tolerated in HIV-infected and uninfected subjects. Although GMTs tended to be lower in the HIV-infected groups, the immune responses in all groups can be linked to surrogate markers of protection against smallpox. The results from this study provide additional valuable data supporting MVA as a promising smallpox vaccine and merit the further investigation of safety and immunogenicity of MVA in HIV-infected subjects with lower CD4+ T-cell counts.

Notes

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