

# Safety Profile of the Viral Vectors of Attenuated Fowlpox Strain FP9 and Modified Vaccinia Virus Ankara Recombinant for Either of 2 Preerythrocytic Malaria Antigens, ME-TRAP or the Circumsporozoite Protein, in Children and Adults in Kenya

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**Background.** We are developing a heterologous prime-boost vaccine strategy against malaria. This approach uses sequential immunization with different vectors to deliver a common preerythrocytic malaria antigen. Preliminary evidence of efficacy and safety has been previously documented in studies from an area where malaria is nonendemic. Additional safety data from an area where malaria is endemic are now required before larger-scale studies are undertaken to determine the efficacy of this vaccine strategy in the field. Other modified vaccinia virus Ankara (MVA) recombinants and prime-boost immunizations are being developed as vaccines against human immunodeficiency virus (HIV) infection, tuberculosis, and cancer, and MVA is a candidate attenuated smallpox vaccine.

**Methods.** Candidate vaccines against malaria were intradermally administered to 73 adults (7 of whom were HIV positive) and 22 children in Kenya. These vaccines used the attenuated fowlpox strain FP9 and the MVA recombinant for either of 2 preerythrocytic malaria antigens, multiple preerythrocytic-stage epitopes joined with the preerythrocytic-stage antigen TRAP (ME-TRAP) and the circumsporozoite protein (CS). Adverse events were recorded.

**Results.** Reactogenicity was mild. MVA caused less frequent and less severe cutaneous reaction if given after FP9 priming. Half doses reduced the frequency and the severity of systemic reactogenicity, and particular vaccine lots were associated with different reactogenicities. Unexpectedly, prior immunity to the ME-TRAP antigen appeared to be protective against local reactions after immunization.

**Conclusions.** Where the final intention is to use MVA after FP9 priming, previous testing of MVA alone overestimates reactogenicity. These recombinant vectors appear to be safe and suitable for use in larger-scale studies of children in Africa and of HIV-positive individuals.

Each year, 1 million deaths are attributed to *Plasmodium falciparum* [1]. We are developing a heterologous prime-boost vaccine [2], using sequential immunization with different vectors to deliver a common preerythrocytic malaria antigen. Both multiple preeryth-

rocytic-stage epitopes joined with the preerythrocytic-stage antigen TRAP (ME-TRAP) and the circumsporozoite protein (CS) have been delivered by the attenuated fowlpox strain (FP9) or modified vaccinia virus Ankara (MVA).

In previous studies performed in The Gambia, 150 adults were immunized with DNA ME-TRAP and then with MVA ME-TRAP [3], 16 adults received FP9 ME-TRAP priming immunizations and then MVA ME-TRAP boost vaccinations [4], and 14 children received a reduced dose ( $3 \times 10^7$  plaque-forming units [pfu] MVA ME-TRAP/mL) (V. S. Moorthy, E. B. Imoukhuede, S. Everaere, S. Keita, M. Pinder, K. Bojang, K.

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McAdam, and A.V.S.H., unpublished data). Systemic and local reactions were similar to those previously observed among subjects in Oxford, United Kingdom [5].

Other MVA recombinants and prime-boost immunizations are being developed as vaccines against HIV infection [6], tuberculosis [7], and cancer. MVA itself is a candidate attenuated smallpox vaccine [8].

The present study reports the first immunizations with FP9 ME-TRAP and MVA ME-TRAP for children, as well as the first immunizations with attenuated poxviruses for HIV-positive adults, in an area where malaria is endemic. The aims of the present study were (1) to establish whether adverse events in this series of phase 1 trials would support further testing, and (2) to describe how reactogenicity varies by sequence of immunization, dose, and antigen insert. We plan to address immunogenicity in a future article (P.B., O. Kai, J. Mwacharo, S. Keating, T.L., S.C.G., N.P., K.M., and A.V.S.H., unpublished data).

## SUBJECTS AND METHODS

**Location.** The study was performed at the Kenya Medical Research Institute, Centre for Geographic Medicine Research (Coast), which is located in Kilifi, Kenya. In Kilifi, an area of endemicity for malaria, transmission of malaria occurs year-round, and there are 2 seasons of high transmission of malaria [9].

**Study design.** The study had an open-label design. If safety data for component vaccines of a regimen were not already available, vaccinations began with half doses, followed by full doses, of the individual component vaccines. These results were reviewed by safety monitors before the complete regimen was used. The safety monitors reviewed all serious adverse events, and their approval was required before additional subjects were immunized. Two safety monitors worked in Kilifi, and a third monitor worked in western Kenya.

Ethical approval was obtained from the Kenyan Medical Research Institute National Ethics Committee and the Oxford University Tropical Research Ethics Committee. Research was conducted in accordance with the Helsinki Declaration of 1975 (which was revised in 1983).

**Study volunteers.** The adults who volunteered to participate in the study worked on local sisal and dairy plantations, were men (to avoid the inclusion of women with an unsuspected pregnancy), and were 18–45 years of age. The children who participated in the study were from the families of the adult workers and were 1–6 years of age; both boys and girls participated.

After public meetings were held and detailed, individual discussions were conducted; written, informed consent was obtained from each adult subject and from a parent or guardian of each child who participated in the study; and a screening

date was suggested. Subjects were immunized no sooner than 1 week after they provided written, informed consent.

Study volunteers were screened on the basis of their medical history and examination and blood test results (i.e., complete blood count, creatinine and alanine transaminase levels, findings on malaria slides, and results of HIV serological tests). Subjects with clinically significant illness were excluded from the study. Subjects with a hemoglobin level of <100 g/L (for adults) or <80 g/L (for children) were also excluded. Subjects with parasitemia were treated with sulfadoxine-pyrimethamine before immunization. HIV rapid tests (Determine; Abbott) were used, and the results were confirmed using the Serozyme ELISA (Adaltis). HIV counseling was offered before testing was performed. Individuals for whom tests results were positive were referred to an HIV clinic and were offered vaccination at a later date, with the exception of individuals who had stage 3 disease. Prevacination viral loads were 600–500,000 RNA copies/mL, and CD4 cell counts were  $1 \times 10^3$  to  $644 \times 10^3$  cells/mL. Only HIV-negative children were immunized.

**Vaccines.** The antigen inserts used were TRAP (joined to a multiple epitope [ME] string from 6 *P. falciparum* preerythrocytic antigens [10]) and CS. The ME string contains 14 preerythrocytic major histocompatibility complex class I epitopes, 3 major histocompatibility class II epitopes, 2 preerythrocytic B cell epitopes, and pb9 (a *Plasmodium berghiei* T cell epitope that allows preclinical potency and stability testing). CS is coupled to the *P. falciparum* class I epitope ls6 (from liver stage antigen 1) and to pb9.

The vectors used were an attenuated fowlpox virus (FP9) and MVA. Recombinant vaccine stock was supplied to contract manufacturer IDT, which produced clinical lots under Good Manufacturing Practices conditions.

Two different batches of FP9 ME-TRAP were used. Batch 020602 was used to immunize 33 adults, and batch 040803 was used to immunize 18 adults and all of the children. Single batches of FP9 CS (batch 010703), MVA CS (batch 020703) and MVA ME-TRAP (batch 031099) were used.

Vaccines were stored at  $-80^{\circ}\text{C}$  and were transported in cool boxes for use within 6 h of storage. Vaccines were administered intradermally over the deltoid area of the nondominant arm, by use of a 27-gauge needle, to create a visible intradermal bleb. Volunteers were observed for 1 h after immunization, with advanced resuscitation facilities available. Children received vitamin A supplements, as per the guidelines of the government of Kenya. The immunization regimens that were used are presented in table 1.

**Assessment of safety.** The same 2 field workers made observations throughout the study. They saw subjects daily for 3 days after vaccination and were supervised by a medically qualified investigator for the first 50 visits. Unsupervised observations were checked by digital photographs taken in the field.

**Table 1. Immunization regimens used in the present study.**

Subject group, immunization regimen <sup>a</sup>	Antigen insert	No. of study volunteers
HIV-negative adults		
FFM	ME-TRAP	10
FM	ME-TRAP	13
FMF	ME-TRAP	5
MFM	ME-TRAP	5
M	ME-TRAP	3
F	CS	3
F	CS	3
M	CS	3
M	CS	3
FFM	CS	6
FM	CS	6
F_M <sup>b</sup>	CS	6
HIV-positive adults		
F	ME-TRAP	2
M	ME-TRAP	2
F	ME-TRAP	1
M	ME-TRAP	2
Children		
Fm	ME-TRAP	2
FM	ME-TRAP	2
Ffm	ME-TRAP	6
ffM	ME-TRAP	6
FFM	ME-TRAP	6

**NOTE.** f, half-dose attenuated fowlpox strain (FP9) immunization (i.e.,  $5 \times 10^7$  plaque-forming units [pfu]/mL, for immunization with FP9 multiple preerythrocytic-stage epitopes joined with the preerythrocytic-stage antigen TRAP [ME-TRAP] and for FP9 circumsporozoite protein [CS]); F, full-dose FP9 immunization (i.e.,  $1 \times 10^8$  pfu/mL, for immunization with FP9 ME-TRAP and for immunization with FP9 CS); m, half-dose modified vaccinia virus Ankara (MVA) immunization (i.e.,  $7.5 \times 10^7$  pfu/mL, for immunization with MVA ME-TRAP, or  $5 \times 10^7$  pfu/mL, for immunization with MVA CS); M, full-dose MVA immunization (i.e.,  $1.5 \times 10^8$  pfu/mL, for immunization with MVA ME-TRAP, or  $1 \times 10^8$  pfu/mL, for immunization with MVA CS).

<sup>a</sup> Abbreviations denote the sequence of vaccination(s) in each regimen (e.g., "ffM" denotes administration of 2 half doses of FP9 [given sequentially], followed by administration of a full dose of MVA). Vaccination intervals were 3 weeks for the regimens using ME-TRAP and 4 weeks for the regimens using CS.

<sup>b</sup> The underscore (\_) denotes an 8-week interval between immunizations.

The medically qualified investigator also saw subjects at 1 week and at 2–3 months after vaccination. Solicited adverse events were recorded, and the diameters of areas of skin discoloration and blistering were measured. Loss of the epidermis or the upper part of the dermis was reported as a "deroofed blister." Unplanned assessments could be requested, and additional weekly visits were made until persistent side effects resolved.

Symptoms were graded according to their influence on activities of daily living. Symptoms were considered to be "mild" if they were without influence, "moderate" if an activity could only be completed with difficulty, and "severe" if an activity of daily living was prevented. An event was considered to be se-

rious if it was life threatening, caused serious disability, or required that the individual be admitted to the hospital.

Blood tests were repeated 7 days after every immunization and at 2 months and 9 months after the final immunization. For HIV-positive subjects, viral loads and CD4 cell counts were measured before, 1 week after, and 4 weeks after immunization.

**Electron microscopic examination of vaccines.** Samples of FP9 ME-TRAP batches 020602 and 040803 were examined by transmission electron microscopy performed after negative staining. Formvar carbon grids underwent glow-discharge treatment for 30 s before application of vaccine diluted 1:50 in Tris buffer (0.1 mol/L; pH 6.8). The grids were then stained with 2% phosphotungstic acid (pH 7.0) and were examined with an FEI F30 microscope at 300 kV.

**Statistical analysis.** Frequencies of side effects were compared using Fisher's exact test. Blistering was reported on the basis of frequency, and the mean diameter of blisters was noted among individuals with blistering. The blister diameter recorded for each subject was the maximum diameter recorded during the 7-day follow-up. A nonparametric test (the Kruskal-Wallis test) was used to compare the diameters. Log-transformed enzyme-linked immunospot assay data were analyzed using regression models.

## RESULTS

Eighty-nine adults and 23 children underwent screening. Five adults (who had either active pulmonary tuberculosis [1 case], chronic renal failure [1 case], orchitis [1 case], or anemia [2 cases]) and 1 child (with relapsing nephrotic syndrome) were excluded from the study. Sixty-six HIV-negative adults were randomly selected and were offered immunization. Six of these 66 adults withdrew consent and were replaced. Of the 10 HIV-positive adults, 7 opted for immunization. All 22 children continued receiving vaccinations, and all vaccine recipients completed the scheduled immunization regimens.

### Serious Adverse Events

An HIV-positive volunteer was immunized with FP9 ME-TRAP and was admitted to the hospital with a swollen, painful left leg 22 days later. Doppler ultrasound scanning confirmed the presence of iliofemoral deep venous thrombosis. The patient recovered after receiving treatment with intravenously administered heparin and warfarin.

A 4-year-old girl developed fever 10 days after immunization with MVA ME-TRAP and was admitted to the hospital with acidosis and *P. falciparum* parasitemia of 24%. The patient demonstrated no unusual features of the illness, and she was discharged from the hospital after 5 days. No other severe adverse events occurred.

**Table 2. Distribution of mild adverse events, by subject group and vaccination.**

Event	HIV-negative adults				HIV-positive adults	Children	
	F CS (n = 30)	F TRAP (n = 51)	M CS (n = 24)	M TRAP (n = 45)	F/M TRAP (n = 7)	F TRAP (n = 40)	M TRAP (n = 22)
Temperature <sup>a</sup>	0 (0)	2 (4)	0 (0)	2 (4)	0 (0)	1 (3)	5 (23)
Fever <sup>b</sup>	3 (10)	10 (20)	1 (4)	4 (9)	0 (0)	9 (23)	4 (18)
Vomiting	0 (0)	5 (9)	2 (8)	2 (4)	1 (14)	4 (10)	0 (0)
Headache	6 (20)	20 (39)	2 (8)	12 (27)	1 (14)	...	...
Pain <sup>c</sup>	13 (43)	17 (33)	5 (21)	17 (38)	0 (0)	10 (25)	5 (23)
Itching <sup>c</sup>	10 (33)	26 (51)	10 (42)	20 (44)	3 (43)	4 (10)	2 (9)
Discoloration diameter, mean cm (95% CI)	0.65 (0.57–0.73)	0.67 (0.62–0.73)	0.7 (0.60–0.82)	0.88 (0.78–1.00)	0.63 (0.44–0.90)	0.36 (0.29–0.44)	0.85 (0.38–1.31)
Intact blister							
Frequency	8 (26)	9 (18)	4 (17)	2 (4)	1 (14)	8 (20)	5 (23)
Diameter, <sup>d</sup> mean cm	0.25	0.21	0.3	0.2	0.2	0.23	0.24
Deroofed blister							
Frequency	7 (23)	9 (18)	6 (25)	1 (2)	0 (0)	9 (23)	3 (13)
Diameter, <sup>d</sup> mean cm	0.25	0.36	0.3	0.2	0 (0)	0.27	0.26
Limited arm motion	1 (3)	1 (1)	1 (4)	0	0	0	0

**NOTE.** Data are no. (%) of subjects, unless indicated otherwise. Each vaccination is considered separately. F CS, attenuated fowlpox strain (FP9) encoding the circumsporozoite protein (CS); M CS, modified vaccinia virus Ankara (MVA) encoding CS; F TRAP, FP9 encoding multiple preerythrocytic-stage epitopes joined with the preerythrocytic antigen TRAP (ME-TRAP); F/M TRAP, aggregate results for subjects receiving either FP9 ME-TRAP or MVA ME-TRAP; M TRAP, MVA encoding ME-TRAP.

<sup>a</sup> Temperature of >37.5°C, when assessed. High temperatures were only recorded the day after immunization.

<sup>b</sup> Febrile symptoms were reported, although not necessarily with a recorded temperature.

<sup>c</sup> At the immunization site only.

<sup>d</sup> For subjects who had blisters.

### Moderately Severe Adverse Events

One adult experienced a headache of 3 days' duration after being immunized with MVA ME-TRAP and had difficulty working. One child developed persistent vomiting 2 days after receiving FP9 ME-TRAP. A severe *Trichiuris* infection was concurrently identified and treated, and the vomiting resolved after 4 days.

### Laboratory Safety Analysis

There were no clinically significant abnormalities in the complete blood count or the findings of biochemical analysis of blood tests during the trial. Among HIV-positive subjects, the median viral load was  $1.1 \times 10^5$  RNA copies/mL (95% CI,  $1.0 \times 10^5$  to  $3.8 \times 10^5$  RNA copies/mL) before vaccination,  $1.4 \times 10^5$  RNA copies/mL (95% CI,  $0.7 \times 10^5$  to  $5.8 \times 10^5$  RNA copies/mL) 1 week after vaccination, and  $1.8 \times 10^5$  RNA copies/mL (95% CI,  $0.6 \times 10^5$  to  $6.3 \times 10^5$  copies/mL) at 1 month after vaccination. The mean CD4 cell count was  $231 \times 10^3$  cells/mL (95% CI,  $60 \times 10^3$  to  $400 \times 10^3$  cells/mL) before vaccination,  $194 \times 10^3$  cells/mL (95% CI,  $56 \times 10^3$  to  $330 \times 10^3$  cells/mL) at 1 week after vaccination, and  $127 \times 10^3$  cells/mL (95% CI,  $50 \times 10^3$  to  $320 \times 10^3$ ) at 1 month after vaccination. The viral load and the CD4 cell count did not change significantly over time ( $P = .2$  to  $P = .5$ , by signed rank test for the various possible comparisons).

### Mild Adverse Events

**Local reactions.** All vaccinations caused local skin discoloration. Erythema and slight cutaneous swelling began within 24 h of immunization. This resolved to leave slightly hyperpigmented areas after 4–7 days. In some study volunteers, blistering began on day 2 or 3 after immunization, and deroofed blisters were noted on day 3 or 4 (median duration of blistering, 2 days for both deroofed and intact blisters). Deroofed blisters were shallow, with sloping edges, and there was a significant association between deroofed and intact blisters ( $P < .0005$ , by Fisher's exact test). The presence of either intact or deroofed blisters was not significantly associated with pain or itching ( $P = .1$ –.26, for the different associations). Hypertrophic scars or keloid reactions were not observed.

The largest deroofed blister was 1 cm in diameter and lasted 4 days (it occurred after immunization with MVA CS without a priming immunization); however, this blister was not painful. Most of these lesions healed rapidly. Seventeen deroofed blisters lasted 1 day only, and 16 healed within 5 days; however, 2 deroofed blisters persisted for 14 days. All local side effects were graded as mild in severity. Thirty-four subjects experienced pain for 1 day, 15 subjects had pain for 2 days, and 3 subjects had pain for 3 days.

**Systemic reactions.** Febrile symptoms occurred frequently (in 12% of adults and in 21% of children), but a measured temperature of >37.5°C was noted less frequently (in 2.5% of

**Table 3. Distribution of mild adverse events, by subject group and sequence of vectors of vaccination.**

Event	Adults					Children	
	F (n = 53)	FF (n = 16)	FM (n = 10)	M (n = 18)	MF (n = 38)	F (n = 22)	FF (n = 18)
Temperature <sup>a</sup>	1 (2)	1 (6)	0 (0)	0 (0)	1 (3)	1 (5)	0 (0)
Fever <sup>b</sup>	8 (15)	2 (13)	3 (30)	2 (11)	2 (5)	8 (36)	1 (6)
Vomiting	4 (8)	0 (0)	1 (10)	1 (6)	2 (5)	3 (14)	1 (6)
Headache	15 (28)	4 (25)	7 (70)	5 (28)	6 (16)	0 (0)	0 (0)
Pain <sup>c</sup>	25 (47)	4 (25)	1 (10)	9 (50)	5 (13)	8 (36)	2 (11)
Itching <sup>c</sup>	25 (47)	4 (25)	3 (30)	11 (61)	13 (34)	4 (18)	0 (0)
Discoloration diameter, mean cm (95% CI)	0.7 (0.64–0.77)	0.58 (0.50–0.65)	0.63 (0.51–0.79)	0.99 (0.79–1.26)	0.75 (0.66–0.86)	0.37 (0.27–0.45)	0.31 (0.24–0.40)
Intact blister							
Frequency	12 (22)	5 (31)	0 (0)	4 (22)	1 (3)	3 (14)	5 (28)
Diameter, <sup>d</sup> mean cm	0.24	0.24	...	0.3	0.2	0.18	0.26
Deroofed blister							
Frequency	14 (26)	1 (6)	1 (10)	4 (22)	2 (5)	2 (9)	7 (39)
Diameter, <sup>d</sup> mean cm	0.4	0.2	0.3	0.4	0.2	0.3	0.26
Limited arm motion, %	0	0	0	0	0	0	0

**NOTE.** Data are no. (%) of subjects, unless indicated otherwise. F, the first attenuated fowlpox strain (FP9) vaccination received by a subject; FF, the second FP9 vaccination received by a subject; FM, FP9 vaccination followed by modified vaccinia virus Ankara (MVA) vaccination; M, MVA given without prior FP9 immunization; MF, MVA vaccination followed by FP9 vaccination.

<sup>a</sup> Temperature of >37.5°C, when assessed. High temperatures were only recorded the day after immunization.

<sup>b</sup> Febrile symptoms were reported, although not necessarily with a recorded temperature.

<sup>c</sup> At the immunization site only.

<sup>d</sup> For subjects who had blisters.

adults and in 10% of children). The highest recorded temperature was 37.8°C for adults and 38.1°C for children. A total of 15% of adults experienced a headache of 1 day's duration, and 5% had a headache of 2 or 3 days' duration. Two adults had mild headaches that lasted for 1 week (after immunization with FP9 CS and FP9 ME-TRAP). Gastrointestinal symptoms were

seen in 5.6% of subjects, and limitation of arm movement lasted for 1 day for 3 subjects only.

#### Mild Adverse Events Varied by Subject Group and Vaccine

**Vaccine.** The different vector antigen combinations caused similar systemic symptoms but significantly different local re-

**Table 4. Distribution of mild adverse events, by subject group and vaccine dose.**

Event	Children				Adults			
	f (n = 14)	F (n = 8)	m (n = 8)	M (n = 14)	f (n = 5)	F (n = 50)	m (n = 5)	M (n = 8)
Temperature <sup>a</sup>	1 (7)	0 (0)	0 (0)	5 (36)	0 (0)	1 (2)	0 (0)	0 (0)
Fever <sup>b</sup>	4 (29)	4 (50)	2 (25)	2 (14)	0 (0)	8 (16)	0 (0)	1 (13)
Vomiting	2 (14)	1 (13)	0 (0)	0 (0)	0 (0)	4 (8)	1 (20)	0 (0)
Headache	...	...	...	...	0 (0)	15 (30)	0 (0)	3 (38)
Pain <sup>c</sup>	7 (50)	1 (13)	2 (25)	3 (21)	0 (0)	25 (50)	2 (40)	4 (50)
Itching <sup>c</sup>	3 (21)	1 (13)	2 (25)	0 (0)	3 (60)	25 (50)	3 (60)	4 (50)
Discoloration diameter, mean cm (95% CI)	0.24 (0.17–0.32)	0.53 (0.32–0.85)	0.39 (0.23–0.66)	0.7 (0.43–1.14)	0.68 (0.3–1.0)	0.74 (0.67–0.81)	1 (0.5–1.4)	1.23 (0.5–2.1)
Intact blister								
Frequency	3 (21)	0 (0)	1 (13)	4 (29)	0 (0)	12 (24)	1 (20)	3 (38)
Diameter, <sup>d</sup> mean cm	0.2	...	0.3	0.225	...	0.24	0.4	0.27
Deroofed blister								
Frequency	2 (14)	0 (0)	2 (25)	1 (7)	0 (0)	14 (28)	2 (40)	2 (25)
Diameter, <sup>d</sup> mean cm	0.2	...	0.3	0.2	...	0.2	0.2	0.15

**NOTE.** Data are no. (%) of subjects, unless indicated otherwise. Data are the aggregate from vectors with either CS or ME-TRAP inserts. f, half-dose attenuated fowlpox strain (FP9) vaccinations; F, full-dose FP9 vaccination; m, half-dose modified vaccinia virus Ankara (MVA) vaccination; M, full-dose MVA vaccination.

<sup>a</sup> Temperature of >37.5°C, when assessed. High temperatures were only recorded the day after immunization.

<sup>b</sup> Febrile symptoms were reported, although not necessarily with a recorded temperature.

<sup>c</sup> At the immunization site only.

<sup>d</sup> For subjects who had blisters.

**Table 5. Distribution of mild adverse events, by batch of attenuated fowlpox strain FP9 ME-TRAP (multiple preerythrocytic-stage epitopes joined with the preerythrocytic-stage antigen TRAP).**

Event	Adults given batch 040803 (n = 18)	Adults given batch 020602 (n = 33)
Temperature <sup>a</sup>	0 (0)	2 (6)
Fever <sup>b</sup>	3 (17)	7 (21)
Vomiting	1 (6)	4 (12)
Headache	8 (44)	12 (36)
Pain <sup>c</sup>	4 (22)	13 (39)
Itching <sup>c</sup>	6 (33)	20 (61)
Discoloration, diameter, mean cm (95% CI)	0.8 (0.66–0.95)	0.61 (0.56–0.66)
Blister		
Frequency	6 (33)	3 (9)
Diameter, <sup>d</sup> mean cm	0.22	0.23
Deroofed blister		
Frequency	7 (39)	2 (6)
Diameter, <sup>d</sup> mean cm	0.58	0.1

**NOTE.** Data are no. (%) of subjects, unless indicated otherwise. Data are for adults only, and batch 020602 was not used in children.

<sup>a</sup> Temperature of >37.5°C, when assessed. High temperatures were only recorded the day after immunization.

<sup>b</sup> Febrile symptoms were reported, although not necessarily with a recorded temperature.

<sup>c</sup> At the immunization site only.

<sup>d</sup> For subjects who had blisters.

actions (table 2). There was more widespread discoloration after immunization with MVA ME-TRAP ( $P = .0015$ ), but immunization with MVA CS caused more deroofed ( $P = .025$ ) and intact ( $P = .052$ ) blisters.

**Subject group.** Side effect profiles were similar for adults and children, except that children more frequently had fever recognized by a measured temperature ( $P = .008$ , only after immunization with MVA), less itching ( $P = .004$ ), and less skin discoloration ( $P = .0001$ ). There were no differences in local or systemic reactogenicity between HIV-positive and HIV-negative subjects, with the exception of a trend for pain developing less frequently among HIV-negative subjects ( $P = .047$ ).

### Sequence of Immunization Modified Cutaneous Reactions

**Local reactions associated with MVA.** When MVA was given without prior FP9 immunization, more widespread discoloration ( $P = .03$ ), intact blisters ( $P = .015$ ), and deroofed blisters ( $P = .04$ ) developed. In 1 subject, immunization with MVA without prior immunization with FP9 resulted in the development of a deroofed blister that was 1 cm in diameter. The largest such blister that occurred after immunization with MVA primed by FP9 was 0.3 cm in diameter. These deroofed blisters healed after 1 or 2 days, but such blisters that occurred after

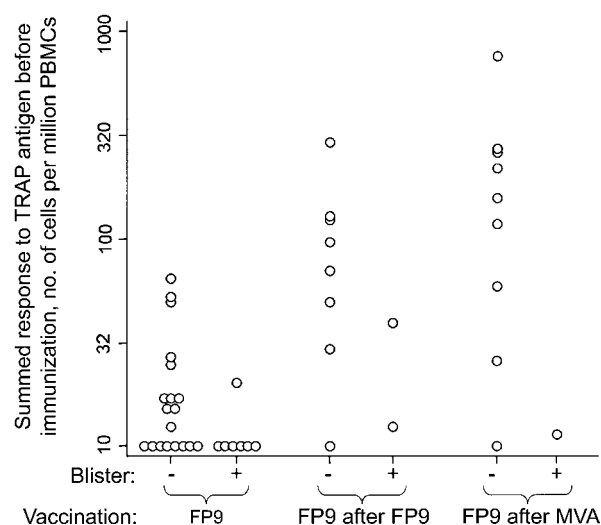
immunization with MVA alone persisted for 14 days in 2 subjects.

**Local reactions associated with FP9.** Similarly, when FP9 immunization was given without prior immunization with FP9 or MVA, discoloration was greater ( $P = .018$ ), although other differences were marginal ( $P = .04$ , for more frequent pain after immunization with FP9 alone;  $P = .087$ , for deroofed blisters) or of little significance ( $P = .24$ , for itching). The distribution of mild adverse events, according to subject group and sequence of vaccination, is detailed in table 3.

**Systemic effects.** Systemic effects occurred independently of the sequence of immunization. FP9 immunization after MVA immunization caused headache more frequently than did either FP9 immunization after prior FP9 immunization or FP9 immunization alone ( $P = .024$ ), but this finding was not corroborated by other systemic symptoms. In children, fever was less commonly reported after the second FP9 immunization than after the first ( $P = .02$ ).

### Adverse Events Varied by Vaccine Dose

Because the vaccination sequence modified reactions, dose comparisons were restricted to the first vaccination (table 4). In adults, systemic symptoms were reduced when half doses were administered ( $P = .04$ , for headache;  $P = .18$ , for fever), but local reactions were similar. In children, immunization with high-dose MVA ME-TRAP was followed by the development of a temperature of >37.5°C in 5 of 14 children the next day but in 0 of 8 children after immunization with half-dose MVA ME-TRAP ( $P = .076$ ). Skin discoloration was greater after im-



**Figure 1.** Individual prevaccination responses to the TRAP antigen, by the sequence of immunization and the presence or absence of blistering. FP9, attenuated fowlpox strain FP9; MVA, modified vaccinia virus Ankara; –, negative for blistering; +, positive for blistering.

**Table 6. Frequency of adverse events, by occurrence after a single immunization or after each immunization.**

Event	Frequency, by comparison of first and second immunizations (n = 73)			Frequency, by comparison of second and third immunizations (n = 44)		
	After first immunization only	After second immunization only	After both immunizations	After second immunization only	After third immunization only	After both immunizations
Temperature <sup>a</sup>	2	2	1	2	1	0
Headache	13	7	6	7	6	3
Vomiting	4	3	1	4	2	0
Fever <sup>b</sup>	11	7	3	7	5	0
Deroofed blister <sup>c</sup>	14	2	5 <sup>d</sup>	5	4	0
Intact blister <sup>c</sup>	11	2	5 <sup>d</sup>	2	8	1
Itching <sup>e</sup>	15	12	11	7	5	6
Pain <sup>e</sup>	21	10	9	9	9	1

**NOTE.** Data are no. of events.

<sup>a</sup> Temperature of >37.5°C, when assessed.

<sup>b</sup> Febrile symptoms were reported, although not necessarily with a recorded temperature.

<sup>c</sup> Among subjects with blistering.

<sup>d</sup>  $P < .05$ .

<sup>e</sup> Occurred at the immunization site only.

munization with high doses, compared with low doses ( $P = .004$ , for FP9;  $P = .06$ , for MVA), but the frequency and severity of the blisters were similar.

#### Batches of FP9 ME-TRAP Had Different Reactogenicity

FP9 ME-TRAP batch 020602 caused less discoloration ( $P = .0078$ ) and fewer intact blisters ( $P = .036$ ) and deroofed blisters ( $P = .0019$ ) than did FP9 ME-TRAP batch 040803 (table 5). On transmission electron microscopic examination, both batches contained discrete viral particles and smaller fragments of membrane, but there were no clear differences between the batches.

#### Prevaccination Immunity to the Antigen Insert Reduced Local Reactogenicity

There was no association between subsequent immune response to the vector or insert and local cutaneous reactions. However, the prevaccination response to the antigen insert was associated with less blistering (figure 1). The geometric mean response was 30 IFN- $\gamma$ -producing cells/million PBMCs (95% CI, 23–39 IFN- $\gamma$ -producing cells/million PBMCs) among subjects who eventually developed blisters, compared with 17 IFN- $\gamma$ -producing cells/million PBMCs (95% CI, 11–25 IFN- $\gamma$ -producing cells/million PBMCs) among subjects who did not develop blisters ( $P = .022$ , by Student's  $t$ -test). There was no difference among responses to the vector before immunization.

The differences in the responses to the inserts were seen only in the recipients of ME-TRAP vaccine ( $P = .0052$ ), rather than in recipients of CS vaccine ( $P = .4$ ). When a regression model was used to adjust for the sequence of immunization (a possible

confounder), a response that was 2.3 times greater than that seen among subjects without blistering was noted ( $P = .018$ ).

#### Local Adverse Events Tended to Recur in Individual Subjects

Seventy-three subjects were immunized more than once. Table 6 presents the frequency with which adverse events recurred. Systemic symptoms were randomly distributed, but if blistering occurred after the first immunization, it was more likely to recur after the second immunization ( $P = .01$ , for intact blisters;  $P = .005$ , for deroofed blisters).

## DISCUSSION

The majority of vaccine recipients experienced mild subjective discomfort but no serious reactogenicity. There was no plausible association between the 2 serious adverse events and immunization, and the safety monitors considered these events to be unrelated to vaccination. Both were isolated events and were not considered to be unusual in the respective subject groups. Deep venous thrombosis is associated with HIV infection [11].

At this preliminary stage, FP9 ME-TRAP and MVA ME-TRAP appear to be safe for use in adults with HIV infection, and there was no evidence that the viral load or the CD4 cell count was adversely affected by vaccination. This is critical, because, in many populations, large-scale HIV testing performed before immunization will be problematic, and a transient increase in the viral load and a decrease in the CD4 cell count have been seen after immunization with pneumococcal and influenza vaccines [12, 13]. There was no evidence that adverse events occurred more frequently or were more severe

among individuals with lower CD4 cell counts and higher viral loads, although there were few adverse events recorded in this group. Compared with adults, children had only minor differences in local skin discoloration. There was some evidence of more frequent objective fever but less frequent symptomatic fever.

MVA CS had a different side effect profile than did MVA ME-TRAP, causing less extensive skin discoloration but more frequent blistering. The viral titer of MVA CS was higher than that of MVA ME-TRAP ( $1 \times 10^9$  pfu/mL vs.  $3 \times 10^8$  pfu/mL). Because the average diameter of blistering was smaller (0.34 cm) than that of discoloration (0.72 cm), this suggests that virus given in a larger volume might disperse more after immunization, causing more-extensive skin discoloration but resulting in less-concentrated virus and therefore causing less frequent blistering.

**Dose and sequence.** Both systemic and local reactogenicities were less severe and less frequent when lower doses of vaccines were administered, although few adult subjects were immunized with lower doses of vaccine. However, the local reactogenicity of MVA was markedly less severe and less frequent if FP9 was given first. Therefore, when the intention is to use MVA as a boosting agent after FP9 in the final regimen, single-dose immunizations should not be assessed first. Dose titration should start with the complete regimen at half dose, and then the switch to a full dose should be made if reactogenicity is acceptable. Such an approach was followed for pediatric vaccinations in the present study.

There was evidence of variable susceptibility of subjects to cutaneous reactions. Subjects who experienced blistering after the first immunization were likely to experience a similar reaction after the second immunization. These host factors might differ by population. If so, this would support conducting phase 1 studies in new populations before larger-scale trials are performed.

**Batch.** Unexpectedly, 2 batches of the same vaccine (FP9 ME-TRAP) were associated with different local reactogenicity. Batches are prepared from the same master seed lot under Good Manufacturing Practices conditions, and Good Manufacturing Practices sterility tests, repeated potency tests, and titrations were performed. The FP9 supplied for the original recombination to generate FP9 ME-TRAP was clonal, having been plaque purified. Differential gene loss during the short growth required to “bulk up” virus during manufacture seems to be unlikely. The batches were of different titers ( $4 \times 10^8$  pfu/mL [for batch 040803] vs.  $1 \times 10^8$  pfu/mL [for batch 020602]). As for the comparison between MVA CS and MVA ME-TRAP, the more concentrated vaccine caused more frequent blistering. However, the less reactogenic FP9 ME-TRAP batch (020602) was also more immunogenic (P.B., O. Kai, J. Mwacharo, S. Keating, T.L., S.C.G., N.P., K.M., and A.V.S.H., unpublished

data), and this finding suggests a more fundamental difference. Because the 2 batches appeared to be similar on electron microscopic examination, the different reactogenicity was not the result of differences in chick fibroblast debris or viral particle aggregation.

**Immune response.** Local cutaneous reactions have been associated with immunogenicity after vaccination with bacille Calmette-Guérin [14], but this was not the case in the present study. However, prior immunity to the malaria antigen insert protected against blistering ( $P = .0052$ ). This finding was only seen among subjects in the subgroup receiving FP9 ME-TRAP, probably because vaccines that contained circumsporozoite protein were less immunogenic (P.B., O. Kai, J. Mwacharo, S. Keating, T.L., S.C.G., N.P., K.M., and A.V.S.H., unpublished data), and blistering was rare after MVA ME-TRAP. The effect was robust to adjustment for vaccination sequence ( $P = .018$ , 2.3-fold difference) (figure 1).

After natural exposure, CS peptides induce IL-10 [15], and MVA induces T regulatory responses (H. A. Fletcher, A. A. Pathan, S. M. Keating, T. K. Berthoud, S. Dunachie, K. T. Whelan, C. R. Sander, A.V.S.H., and H. McShane, unpublished data). It is thus possible that TRAP-specific responses down-regulate local inflammation after vaccination.

In conclusion, the vaccine regimens employed in the present study were found to be safe. The data presented detail the determinants of reactogenicity and inform the conduct of future phase 1 studies.

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