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ORIGINAL ARTICLE

Phase 3 Efficacy Trial of Modified Vaccinia Ankara as a Vaccine against Smallpox

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Abstract

BACKGROUND

Many countries have stockpiled vaccines because of concerns about the reemergence of smallpox. Traditional smallpox vaccines are based on replicating vaccinia viruses; these vaccines have considerable side effects.

METHODS

To evaluate the efficacy of modified vaccinia Ankara (MVA) as a potential smallpox vaccine, we randomly assigned 440 participants to receive two doses of MVA followed by one dose of the established replicating-vaccinia vaccine ACAM2000 (the MVA group) or to receive one dose of ACAM2000 (the ACAM2000-only group). The two primary end points were noninferiority of the MVA vaccine to ACAM2000 with respect to the peak serum neutralizing antibody titers and attenuation of the ACAM2000-associated major cutaneous reaction by previous MVA vaccination, measured according to the maximum lesion area and the derived area attenuation ratio.

RESULTS

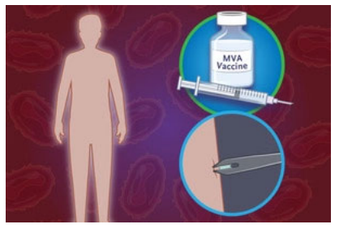
A total of 220 and 213 participants were randomly assigned and vaccinated in the MVA group and ACAM2000-only group, respectively, and 208 participants received two MVA vaccinations. At peak visits, MVA vaccination induced a geometric mean titer of neutralizing antibodies of 153.5 at week 6, as compared with 79.3 at week 4 with ACAM2000 (a ratio of 1.94 [95% confidence interval {CI}, 1.56 to 2.40]). At day 14, the geometric mean titer of neutralizing antibodies induced by a single MVA vaccination (16.2) was equal to that induced by ACAM2000 (16.2), and the percentages of participants with seroconversion were similar (90.8% and 91.8%, respectively). The median lesion areas of the major cutaneous reaction were 0 mm^2 in the MVA group and 76.0 mm^2 in the ACAM2000-only group, resulting in an area attenuation ratio of 97.9% (95% CI,

96.6 to 98.3). There were fewer adverse events or adverse events of grade 3 or higher after both MVA vaccination periods in the MVA group than in the ACAM2000-only group (17 vs. 64 participants with adverse events of grade 3 or higher, P<0.001).

CONCLUSIONS

No safety concerns associated with the MVA vaccine were identified. Immune responses and attenuation of the major cutaneous reaction suggest that this MVA vaccine protected against variola infection. (Funded by the Office of the Assistant Secretary for Preparedness and Response Biomedical Advanced Research and Development Authority of the Department of Health and Human Services and Bavarian Nordic; ClinicalTrials.gov number, NCT01913353.)

Introduction



QUICK TAKE

A Modified Smallpox Vaccine

02:15

The eradication of smallpox through vaccination[1] has not eliminated the risk of reintroduction of the infection[2,3] by accidental or intentional release of the variola virus. Moreover, recent studies have shown the potential for de novo synthesis.[4,5]

Smallpox vaccination was highly successful, but it had toxic effects that could be severe, particularly in populations with immunodeficiencies or skin disorders.[6-10] These effects are related to the replication competence of the vaccinia virus used in vaccination. Modified vaccinia Ankara (MVA) is being developed as a potentially safer smallpox vaccine. MVA was identified after 570 passages of the chorioallantois vaccinia Ankara strain through chicken embryo fibroblast cells.[11] In the 1970s, MVA was administered before traditional smallpox vaccination to more than 120,000 persons, including children with immunodeficiency, to improve safety.[12] More recently, it has been evaluated as a stand-alone vaccine that has produced immune responses that are similar to those of traditional smallpox vaccines and has an improved safety profile.[13-21]

Replication-competent vaccines are administered by means of intradermal scarification, resulting in a major cutaneous reaction,[22] or "take," which is considered to be an indicator of protection against smallpox. MVA does not cause major cutaneous reactions because of a lack of replication in human cells.[23] A reduction in the size or rate of the major cutaneous reaction has been linked to a protective immune response that neutralizes replication-competent vaccines.[24]

The true efficacy of vaccines to prevent smallpox can no longer be evaluated in the field, so clinical trials evaluating these vaccines must rely on efficacy correlates. On the basis of a precedent from the approval of the established replicating-vaccinia vaccine ACAM2000,[22,25,26] and in agreement with the Food and Drug Administration (FDA), we defined the two primary efficacy end points of this trial as a comparison of neutralizing antibody titers induced by MVA and ACAM2000 and the attenuation of the major cutaneous reaction by previous vaccination with MVA.

Because of its potential toxic effects, there are few populations in which ACAM2000 is typically administered. One population with sufficient risk to justify vaccination is soldiers deployed to U.S. military garrisons in South Korea, which was the chosen site for this trial.

Methods

TRIAL DESIGN AND OVERSIGHT

In this phase 3, open-label, randomized trial, MVA was compared with ACAM2000, and two indicators of efficacy for protection against smallpox were assessed. The two primary end points were immunogenicity (noninferiority of the MVA vaccine to ACAM2000 with respect to the peak geometric mean titer of serum neutralizing antibodies) and a surrogate of efficacy (attenuation of the major cutaneous reaction by previous

vaccination with MVA, measured as the maximum lesion area, after receipt of ACAM2000). A total of 440 healthy participants who had not previously received any vaccinia-based vaccine were eligible for enrollment and randomization, in a 1:1 ratio, to one of the two vaccination groups. The MVA group received two doses of MVA, one each on weeks 0 and 4, followed by ACAM2000 on week 8. The ACAM2000-only group received ACAM2000 on week 0.

Safety and reactogenicity, including solicited local and general adverse events, as well as unsolicited adverse events, were assessed throughout the active trial phase. Cardiac monitoring was performed until 6 months after the last vaccination in both the MVA group and the ACAM2000-only group.

The trial was designed by Bavarian Nordic on the basis of comments and feedback received from the FDA. Trial data were collected and analyzed by the U.S. Army Medical Research Institute of Infectious Diseases, Bavarian Nordic, and Chiltern International, a contract research organization. All the authors vouch for the completeness and accuracy of the data and analyses and for the fidelity of the trial to the protocol (available with the full text of this article at NEJM.org). The manuscript was prepared by authors who are employees of Bavarian Nordic. The conduct of the trial was managed by Bavarian Nordic and Chiltern. The trial was conducted at a U.S. military garrison in South Korea led by the U.S. Army Medical Research Institute of Infectious Diseases in collaboration with the Defense Health Agency.

The trial was conducted in accordance with the Declaration of Helsinki and the Belmont Report and was approved by the institutional review boards of the U.S. Army Medical Research Institute of Infectious Diseases. All the participants provided written informed consent.

PARTICIPANTS

Participants were enrolled at the Brian Allgood Army Community Hospital at Yongsan U.S. Military Garrison and Camp Hovey, both in South Korea. They were recruited from Department of Defense personnel who were scheduled to receive an initial smallpox vaccination as part of their in-process orientation.

Healthy persons who were 18 to 42 years of age were eligible to participate if they had a body-mass index (the weight in kilograms divided by the square of the height in meters) between 18.5 and 34.9, a negative serum pregnancy test, a white-cell count of 2500 to 10,999 per cubic millimeter, normal hemoglobin and platelet levels, normal renal and hepatic function, a troponin I level that was less than 2 times the upper limit of the normal range, and echocardiographic (ECG) tests without clinically significant findings (normal laboratory ranges are provided in the Supplementary Appendix, available at NEJM.org).

Participants were excluded if they had a known or suspected history of smallpox-based or poxvirus-based vaccination, a history of or active immunodeficiency or immune suppression or close contact with a person with immunodeficiency, a history of certain heart conditions, a high risk of death from cardiovascular causes, or certain active skin conditions or a history of such conditions.

VACCINE

MVA was produced at Bavarian Nordic (Kvistgård, Denmark) and filled, formulated, and labeled at IDT Biologika (Dessau-Roßlau, Germany). A standard 0.5-ml dose of liquid frozen vaccine had a nominal titer of 1×10^8 $TCID_{50}$ (50% tissue-culture infectious dose). MVA was injected subcutaneously into the upper arm.

ACAM2000 vaccine was supplied by the Defense Health Agency. Department of Defense personnel administered the vaccine through scarification with a bifurcated needle (standard dose, 2.5×10^5 to 12.5×10^5 plaque-forming units of live vaccinia virus) into the upper arm.

ASSESSMENTS OF IMMUNOGENICITY

Serum antibody titers were measured with the use of a plaque-reduction neutralization test (PRNT) and an enzyme-linked immunosorbent assay (ELISA). The vaccinia-specific PRNT and ELISA used the vaccinia virus Western Reserve strain and MVA, respectively, as antigens; the detection limits were titers of 2 and 50, respectively.

ASSESSMENTS OF THE MAJOR CUTANEOUS REACTION

The Silhouette Connect camera system was used to photograph the major cutaneous reaction and calculate the lesion area (Fig. S1 in the Supplementary Appendix). The maximum lesion area was defined as the larger area measured once during days 6 through 8 and once during days 13 through 15 (relative to scarification). An independent review committee consisting of three assessors who were unaware of the trial-group assignments confirmed or updated the lesion area measurements (Fig. S2). Using photos of the lesions taken on one day during each of the two periods (days 6–8 and 13–15), the committee also evaluated, in blinded fashion, each lesion as full (major lesion diameter, ≥5 mm), partial, or without a major cutaneous reaction (at days 6–8 after scarification). The diameter of the major lesion, major erythema, and major induration were assessed by an investigator twice (at days 6–8 and 13–15). The committee also reviewed local symptom data from participants on printed diary cards through 14 days after scarification.

STATISTICAL ANALYSIS

We specified that statistical significance at a type I error of 0.05 would be needed for both primary end points. No adjustment was made for multiple comparisons, and the 95% confidence intervals were not adjusted. All tests and confidence intervals are two-sided. The sample size provided at least 80% power to assess both primary end points. Efficacy analyses were conducted in the per-protocol population, the full-analysis population, and in all participants who underwent randomization, with the per-protocol population being the primary analysis population. Safety analyses were based on the full-analysis population (participants who underwent randomization and were vaccinated).

The first primary end point was the noninferiority of MVA to ACAM2000 with respect to the geometric mean titer of neutralizing antibodies at peak visits (week 6 in the MVA group and week 4 in the ACAM2000-only group, according to published data).[15,18,25] The noninferiority of MVA to ACAM2000 was shown if the 95% confidence interval of the geometric mean titer ratio as measured by PRNT (MVA:ACAM2000-only, obtained by calculating the antilog of the difference in the mean $\log_{10}$-transformed titers) was entirely above 0.5. Secondary immunogenicity end points included seroconversion and geometric mean titers at each visit determined with the use of PRNT and ELISA. Seroconversion was defined as seropositivity after baseline (in patients who were seronegative at baseline) or two or more times the baseline titer (in patients who were seropositive at baseline).

The second primary end point was the attenuation of a major cutaneous reaction when ACAM2000 was administered after previous MVA vaccination, as determined by the maximum lesion area confirmed by the independent review committee. The goal was an area attenuation ratio (1 – the maximum lesion area ratio [MVA:ACAM2000-only]) that was significantly above 40%. The maximum lesion area ratio and 95% confidence interval were based on the Hodges–Lehmann estimate of the shift for log-transformed maximum lesion areas. Secondary efficacy end points included evaluation of the maximum lesion diameter and the lesion diameter measured by the investigator twice (at days 6–8 and 13–15) as well as classification of the major cutaneous reaction as full, partial, or absent.

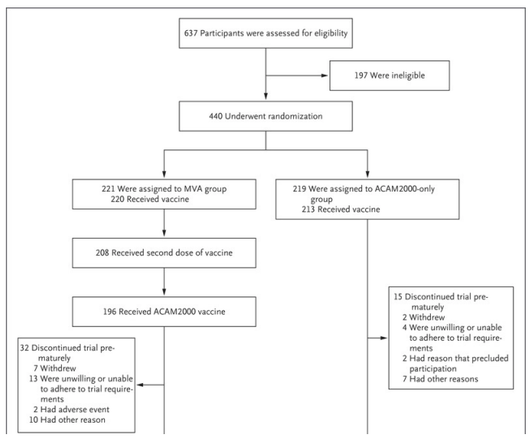
A safety analysis, including the number and percentage of participants who had adverse events, was performed during each vaccination period. There were three vaccination periods in the MVA group (period 1 [weeks 0–4], period 2 [weeks 4–8], and period 3 [weeks 8–12]) and one vaccination period in the ACAM2000-only group (period 1 [weeks 0–4]). Each period in the MVA group was compared with the period in the ACAM2000-only group by means of Fisher's exact test.

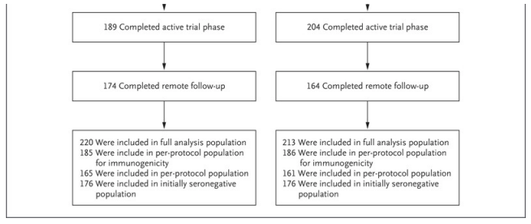
Randomization was performed by a third-party organization using a validated interactive Web portal. This was an open-label trial, but the independent review committee and personnel responsible for immunogenicity testing were unaware of the trial-group assignments. Further specific details regarding the trial design are provided in the protocol.

Results

DEMOGRAPHIC AND BASELINE CHARACTERISTICS

Figure 1.





Screening, Randomization, and Follow-up.

Table 1.

Table 1. Demographic Characteristics of the Participants (Full-Analysis Population).*

Characteristic	MVA Group (N=220)	ACAM2000-Only Group (N=213)	All Participants (N=433)
Age — yr	23.5±4.77	23.4±4.58	23.5±4.67
Sex — no. (%)			
Female	39 (17.7)	29 (13.6)	68 (15.7)
Male	181 (82.3)	184 (86.4)	365 (84.3)
Height — cm	174.00±9.169	173.87±9.140	173.93±9.144
Weight — kg	79.38±13.463	79.90±14.234	79.64±13.834
Body-mass index	26.12±3.297	26.30±3.292	26.21±3.291
Race or ethnic group — no. (%)†			
American Indian or Alaskan Native	8 (3.6)	6 (2.8)	14 (3.2)
Asian	14 (6.4)	12 (5.6)	26 (6.0)
Black	48 (21.8)	40 (18.8)	88 (20.3)
Native Hawaiian or other Pacific Islander	5 (2.3)	3 (1.4)	8 (1.8)
White	126 (57.3)	136 (63.8)	262 (60.5)
Other race	19 (8.6)	16 (7.5)	35 (8.1)
Hispanic or Latino	54 (24.5)	40 (18.8)	94 (21.7)
Not Hispanic or Latino	166 (75.5)	173 (81.2)	339 (78.3)

* Plus–minus values are means ±SD. Weight and body-mass index (the weight in kilograms divided by the square of the height in meters) were based on the weight at screening. Percentages may not total 100 because of rounding. There were no missing values in any of the demographic characteristics.
† Race and ethnic group were reported by the participants.

Demographic Characteristics of the Participants (Full-Analysis Population).

A total of 440 participants (221 in the MVA group and 219 in the ACAM2000-only group) underwent randomization beginning on March 24, 2015. The last follow-up visit occurred on August 14, 2017; a total of 433 participants were vaccinated (Figure 1). In the MVA group, 220 participants (99.5%) received the first dose of MVA vaccine and 208 participants (94.1%) received the second dose of MVA vaccine; 196 participants (88.7%) in this group also received the ACAM2000 vaccine. A total of 12 participants in the MVA group withdrew from the trial and did not receive the second dose of vaccine (7 were unwilling or unable to comply with visit schedules, 3 withdrew consent, and 2 had adverse events). In the ACAM2000-only group, 213 participants (97.3%) received a single dose of the ACAM2000 vaccine. The two groups were well matched with respect to demographic characteristics (Table 1).

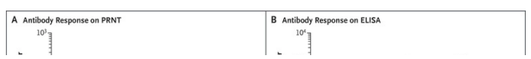
PARTICIPANTS

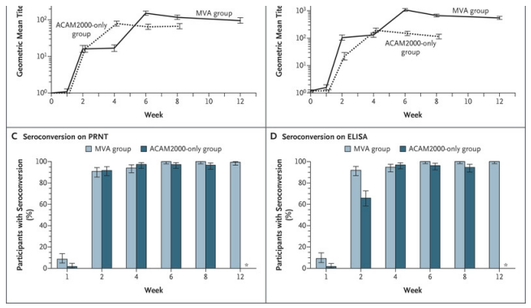
A total of 47 participants (10.7%) prematurely discontinued the trial after randomization (Figure 1). The reasons included participant request, unwillingness or inability to adhere to trial requirements, adverse events, and other reasons (Table S10). In the MVA group, the two adverse events that led to trial withdrawal were severe tibia fracture and moderate nonspecific chest pain; neither was considered by the investigators to be related to MVA.

IMMUNOGENICITY RESULTS

Neutralizing antibodies are considered to be an important factor in protection against smallpox.[27] In the per-protocol population, the geometric mean titer of neutralizing antibodies at peak visits in the MVA group was 153.5 at week 6, which was higher than that of the ACAM2000-only group (79.3 at week 4). This resulted in a geometric mean titer ratio of 1.94 (95% confidence interval [CI], 1.56 to 2.40) and met the threshold of noninferiority for one of the primary end points. Similar results were observed in all participants who underwent randomization when missing data were imputed through multiple imputation, with a peak-visit geometric mean titer of 152.8 in the MVA group and 81.1 in the ACAM2000-only group (geometric mean titer ratio, 1.88; 95% CI, 1.47 to 2.42).

Figure 2.





Antibody Responses and Seroconversion among Participants in the Per-Protocol Population for Immunogenicity.

At peak visits, all the participants in the per-protocol population in the MVA group and 97.3% of the participants in the ACAM2000-only group had seroconversion according to PRNT (Figure 2). At weeks 2 and 4, time points when ACAM2000 has historically been considered to be protective, the percentages of participants with seroconversion were similar in the two groups (Figure 2 and Tables S4 and S5).

Since most participants who received ACAM2000 in the ACAM2000-only group had a full major cutaneous reaction by day 6 through 8, they were considered to be protected against smallpox.[22,24] In the per-protocol population, 1.6% of the participants had seroconversion according to PRNT on week 1, but this percentage increased to 91.8% by week 2 (geometric mean titer, 16.2). Similar kinetics were observed in the MVA group after a single vaccination with MVA. The incidence of seroconversion according to PRNT increased from 8.7% at week 1 to 90.8% at week 2 (geometric mean titer, 16.2) (Figure 2 and Table S4). These results were similar to those in the ACAM2000-only group (geometric mean titer ratio of MVA to ACAM2000-only, 1.00; 95% CI, 0.74 to 1.35). ELISA yielded results that were similar (Figure 2) to those determined by PRNT. Likewise, the geometric mean titers were 14.8 in the MVA group and 15.6 in the ACAM2000-only group, with a ratio of 0.95 (95% CI, 0.68 to 1.32), after imputation of missing data in all participants who underwent randomization.

INCIDENCE OF MAJOR CUTANEOUS REACTIONS AND SURROGATE MARKER OF EFFICACY

Table 2.

Table 2. Assessment of Major Cutaneous Reactions after ACAM2000 Vaccination (Per-Protocol Population).*

Visit and Measurement	MVA Group (N = 165)		ACAM2000-Only Group (N = 161)		Area Attenuation Ratio or Diameter Attenuation Ratio (95% CI)†
	Median (95% CI)	Range	Median (95% CI)	Range	
Lesion area — mm²					
Days 6–8‡	0 (0–1)	0–96	37 (33–42)	0–133	95.2 (93.8–96.2)
Days 13–15	0 (0–0)	0–99	75 (69–85)	0–368	98.2 (97.7–98.4)
Maximum area‡	0 (0–2)	0–99	76 (70–87)	0–368	97.9 (96.6–98.3)
Lesion diameter — mm					
Days 6–8‡	0 (0–2)	0–12	8 (8–9)	0–16	80.0 (77.8–85.7)
Days 13–15	0 (0–0)	0–12	10 (10–11)	0–25	88.9 (87.5–90.0)
Maximum diameter‡	0 (0–2)	0–12	11 (10–11)	0–25	87.5 (83.3–88.9)

* Analyses were based on the per-protocol population. Results from the full-analysis population were similar (Table S8). CI denotes confidence interval.
† The area attenuation ratio is shown for the lesion areas measured by the investigator with the use of the Silhouette Connect camera system and confirmed by an independent review committee that was unaware of the trial-group assignments. The area attenuation ratio is equal to 1 − maximum lesion area ratio (MVA group:ACAM2000-only group). The maximum lesion area ratio and 95% confidence interval are based on the Hodges–Lehmann estimate of the shift for log-transformed lesion areas. Areas of lesions that were not visible were included with a value of 1 mm². Days 6–8 and days 13–15 refer to days 6–8 and days 13–15 after ACAM2000 scarification. The diameter attenuation ratio is shown for lesion diameters measured by the investigator. The diameter attenuation ratio is equal to 1 − lesion diameter ratio (MVA group:ACAM2000-only group). The lesion diameter ratio and 95% confidence intervals are based on the Hodges–Lehmann estimate of the shift for log-transformed lesion diameters. Diameters of lesions that were not visible were included with a value of 1 mm. Days 6–8 and days 13–15 refer to days 6–8 and days 13–15 after ACAM2000 scarification.
‡ Data on lesion area and diameter were not available for one participant at the days 6–8 visit in the MVA group, and the maximum area and diameter are reported for 164 participants in that group.

Assessment of Major Cutaneous Reactions after ACAM2000 Vaccination (Per-Protocol Population).

In the per-protocol population, the median maximum lesion areas in the MVA group and ACAM2000-only group were 0 mm^2 (95% CI, 0 to 2.0) and 76.0 mm^2 (95% CI, 70.0 to 87.0), respectively. These results showed a significant area attenuation ratio that exceeded the predefined 40% threshold (Table 2) and met this primary efficacy end point. The area attenuation ratio of 97.9% (95% CI, 96.6 to 98.3) showed that receipt of MVA before ACAM2000 scarification resulted in attenuation of the major cutaneous reaction.

Similar results were observed in the group of all participants who underwent randomization, with a median maximum lesion area of 0 and 75.0 mm^2 in the MVA and ACAM2000-only groups, respectively (Table S8). These results were supported by the maximum lesion diameter as measured by the investigators (Table 2) and the assessments by the independent review committee regarding major cutaneous reactions, which indicated that the MVA group had substantially fewer full major cutaneous reactions (23.0% vs. 92.5%) and more partial major cutaneous reactions (23.0% vs. 4.3%) and absent major cutaneous reactions (53.9% vs. 1.9%) (Tables S1 and S9). When missing data were imputed by the worst-case scenario (full major cutaneous reaction in the MVA group and absent major cutaneous reaction in the ACAM2000-only group), the

percentage of participants with a full major cutaneous reaction was 31.7% and the percentage without a major cutaneous reaction was 48.9% in the MVA group; in the ACAM2000-only group, these percentages were 87.7% and 8.2%, respectively.

SAFETY AND REACTOGENICITY RESULTS

Table 3.

Table 3. Pooled Solicited and Unsolicited Adverse Events during the Active Trial Phase in Each Vaccination Period (Full-Analysis Population).*

Event	MVA Group							ACAM2000-Only Group
	Period 1 MVA (N=220)	P Value†	Period 2 MVA (N=208)	P Value†	Periods 1 and 2 MVA (N=220)	Period 3 ACAM2000 (N=196)	P Value†	Period 1 (N=213)
	no. (%)		no. (%)		no. (%)			no. (%)
Documented adverse event	169 (76.8)	<0.001	135 (64.9)	<0.001	184 (83.6)	181 (92.3)	0.008	209 (98.1)
Nonserious adverse event within 29 days after vaccination	168 (76.4)	<0.001	135 (64.9)	<0.001	183 (83.2)	181 (92.3)	0.008	209 (98.1)
Serious adverse event‡	2 (0.9)	1.0	0	1.0	2 (0.9)	0	1.0	1 (0.5)
Adverse event of special interest	2 (0.9)	0.44	2 (1.0)	0.68	4 (1.8)	2 (1.0)	0.69	4 (1.9)
Related adverse event within 29 days after vaccination§	112 (50.9)	<0.001	76 (36.5)	<0.001	130 (59.1)	61 (31.1)	<0.001	158 (74.2)
Adverse event grade ≥3 within 29 days after vaccination	13 (5.9)	<0.001	4 (1.9)	<0.001	17 (7.7)	10 (5.1)	<0.001	64 (30.0)
Related adverse event grade ≥3§	3 (1.4)	<0.001	2 (1.0)	<0.001	5 (2.3)	3 (1.5)	<0.001	22 (10.3)
Related adverse event grade ≥3 within 29 days§	3 (1.4)	<0.001	2 (1.0)	<0.001	5 (2.3)	3 (1.5)	<0.001	22 (10.3)
Adverse event leading to withdrawal from trial	2 (0.9)	0.5	0	NA	2 (0.9)	0	NA	0
Adverse event leading to withdrawal from vaccination	2 (0.9)	0.5	0	NA	2 (0.9)	0	NA	0

* Instances in which patients had at least one documented adverse event are listed. There were no deaths during the trial. NA denotes not applicable.
† P values for the comparison of the relevant MVA group vaccination period with the ACAM2000-only group vaccination period were calculated with the use of Fisher's exact test. They are considered to be descriptive only. There may have been findings in more than one category.
‡ Only two of the serious adverse events occurred during the 29-day follow-up period; the serious adverse event in the ACAM2000-only group occurred after the 29-day follow-up period but within the active trial phase because the participant's fourth visit was conducted 15 days later than scheduled.
§ Related adverse events were unsolicited or general solicited adverse events that the investigator considered to be possibly, probably, or definitely related to the trial vaccine or events for which no causality assessment was made by the investigator. All solicited local adverse events were defined as being related to the vaccine and are not included in this category.

Pooled Solicited and Unsolicited Adverse Events during the Active Trial Phase in Each Vaccination Period (Full-Analysis Population).

Safety results were based on the full-analysis population. Overall, there were considerably fewer adverse events or adverse events of grade 3 or higher within 29 days for all three vaccination periods in the MVA group than in the ACAM2000-only group (P≤0.008 for all comparisons) (Table 3). Besides general disorders and administration-site conditions (57% in the MVA group vs. 96% in the ACAM2000-only group), the most common adverse events within 29 days after MVA vaccination were headache (16%), myalgia (14%), and lymphadenopathy (9%), whereas the most common adverse events in the ACAM2000-only group were lymphadenopathy (51%), headache (38%), myalgia (36%), and contact dermatitis (23%) (Table S11). Within 29 days after MVA vaccination, there were 29 adverse events of grade 3 or higher in 4% of the participants who received MVA vaccinations in the MVA group, as compared with 114 events in 30% of the participants in the ACAM2000-only group (Table S12).

SOLICITED ADVERSE EVENTS

All solicited local adverse events (pain, erythema, swelling, induration, and pruritus) were more frequent in the ACAM2000-only group than in the MVA group during any MVA vaccination period and after ACAM2000 vaccination when preceded by MVA vaccination (P<0.001 for all solicited adverse events). Pain was the most prevalent administration-site reaction after MVA vaccination, and erythema and pruritus were the most prevalent administration-site reactions after ACAM2000 vaccination. There were also fewer grade 3 adverse events in the MVA group during the MVA vaccination periods than there were in the ACAM2000-only group (6 vs. 57) (Table S2).

Each of the solicited general adverse events (pyrexia, headache, myalgia, chills, nausea, fatigue, and malaise) occurred more frequently in the ACAM2000-only group than in either MVA period (except pyrexia, which occurred equally in MVA period 1 and the ACAM2000-only group) (Table S3).

UNSOLICITED ADVERSE EVENTS

Approximately half of all vaccinations were followed by at least one unsolicited adverse event (in 60.9%, 46.2%, and 57.1% of the vaccinations in periods 1, 2, and 3 in the MVA group, respectively, and in 55.9% of the vaccinations in the ACAM2000-only group). The proportions of unsolicited adverse events that were considered by the investigators to be at least possibly related to vaccination during the 29-day vaccination periods were similar across periods and between groups. Two related unsolicited adverse events were grade 3 or higher (nonserious events of dyspnea and photosensitivity after ACAM2000 vaccination).

SERIOUS ADVERSE EVENTS AND ADVERSE EVENTS OF SPECIAL INTEREST

Eight serious adverse events were reported, two of which (appendicitis and tibia fracture, in the MVA group after the first MVA vaccination) occurred within the 29-day follow-up period; both were considered by the investigators to be unrelated to vaccination. There were no deaths during the trial, and two adverse events led to withdrawal from vaccination in the MVA group. Ten participants had an adverse event of special interest; four of these events were possibly related to vaccination (two in each group; two events of exertional dyspnea, one event of dyspnea, and one event of noncardiac chest pain). All four events occurred in ACAM2000 vaccination periods. None of the adverse events of special interest were considered by the investigators to be serious, and the investigator did not consider any ECG results or abnormal troponin I values to be clinically significant.

Discussion

This phase 3 trial meets the proposed efficacy criteria for MVA as a smallpox vaccine, as assessed with the use of surrogate efficacy measures. Although more vaccinations and a longer trial duration in the MVA group than in the ACAM2000-only group in this open-label trial design resulted in an imbalance of missing data as a result of early discontinuation in the two groups, the findings have been shown to be consistent according to different methods for imputation of missing data, including the worst-case scenario imputation (Tables S5, S8, and S9). The immune responses with MVA were similar to those with traditional smallpox vaccines, as previously observed,[18,21] and the similar efficacy of MVA and traditional smallpox vaccines has been observed in relevant animal models.[28-31]

Previous MVA vaccination prevented formation of a full major cutaneous reaction in the majority of participants (77.0%) after subsequent ACAM2000 vaccination, as compared with a rate of full major cutaneous reaction of 92.5% after ACAM2000 alone. The maximum lesion area of the major cutaneous reaction was significantly reduced when ACAM2000 vaccination was preceded by MVA vaccination. These results are consistent with the findings observed in persons revaccinated with traditional smallpox vaccines, who were considered to be protected against smallpox on the basis of attenuation of the major cutaneous reaction.[24]

Induction of neutralizing antibodies by smallpox vaccines is considered to be an important immunologic factor in protection against smallpox.[27] Two MVA vaccinations were noninferior to standard ACAM2000 vaccination in inducing peak neutralizing antibody titers. The peak responses occurred at different time points, probably because of differences in administration and the biologic characteristics of the two vaccines. With traditional smallpox vaccines, vaccinia is applied intradermally by scarification, creating a local infection and virus-filled pustule that scabs and leaves a scar. Lesion viral shedding occurs for more than 20 days, with a peak at day 12 to 14 after vaccination.[18,32,33] Consequently, the immune system may be continuously stimulated for 20 days or longer, causing detectable neutralizing antibodies in most participants by day 14 (at peak lesion viral load), and peaking at day 28.[18,26,30,33-35] In contrast, MVA does not replicate in human cells.[23] This provides short-lived immune priming. Thus, early immune responses (at day 14) induced by MVA are similar to those of ACAM2000, but the lack of replication and antigen persistence result in antibody plateau at day 14, and antibodies increase only after MVA booster vaccination is applied in the two-vaccination regimen.

Although the comparison of neutralizing antibody titers at peak visits is considered to be evidence of efficacy of the smallpox vaccine, the absolute antibody titer required for protection against the variola virus is not known. Epidemiologic studies of smallpox index cases showed that vaccination against smallpox prevented disease after subsequent variola exposure even in the absence of measurable neutralizing antibodies.[27,36] With traditional vaccines, the formation of a major cutaneous reaction by days 6 through 8 shows protective immunity against the variola virus.[22,24] However, immune responses are generally not measurable until days 12 through 14 after vaccination.[21,32,34,35] At day 14, the PRNT-measured geometric mean titer induced by one MVA dose is similar to that of ACAM2000. This finding suggests that single MVA vaccination may provide protection that is similar to that of ACAM2000. Only at day 28 after vaccination did ACAM2000 induce a higher neutralizing antibody response than the response with one MVA dose, but no meaningful differences in seroconversion rates as measured by PRNT were observed at week 2 or week 4 after vaccination.

These findings are supported by the observation of more rapid protection after single MVA vaccination than with traditional smallpox vaccines in relevant animal models. MVA vaccination protected nonhuman primates from lethal monkeypox virus challenge within 4 days, as compared with 6 days with traditional vaccines.[31] Similarly, MVA administered on the same day or 2 days after a lethal challenge with ectromelia virus, a variola-related murine virus, fully protected immune-suppressed mice.[28] Traditional smallpox vaccines did not provide the same protection. In both animal models, MVA provided protection despite the absence of detectable neutralizing antibodies, but the animals had been primed, as shown by a rapid immune boost observed after challenge that was not observed in animals that received placebo.

MVA met both primary end points, with a better safety profile than ACAM2000. The peak neutralizing antibody titer after MVA, thought to be a key measure of protection, was higher than that induced by ACAM2000, and MVA significantly reduced the lesion area from ACAM2000 vaccination, a historical surrogate of protection against smallpox. A single MVA vaccination induced neutralizing antibody titers that were similar to those with ACAM2000 at day 14, when protection against variola, as judged by the induction of a vaccine major cutaneous reaction, is apparent. Furthermore, previous studies have shown that MVA can be used in certain immunocompromised populations for which the use of ACAM2000 is contraindicated.[15,16,18,37,38]

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Supplementary Material

Protocol	PDF	14376KB
Supplementary Appendix	PDF	1564KB
Disclosure Forms	PDF	228KB
Data Sharing Statement	PDF	56KB

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R HOLBROOK
Nov 14, 2019

Impossible to understand.
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PAGE 1

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