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A randomized phase II trial to compare safety and immunogenicity of the MVA-BN smallpox vaccine at various doses in adults with a history of AIDS

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ABSTRACT

Traditional replicating smallpox vaccines are associated with serious safety concerns in the general population and are contraindicated in immunocompromised individuals. However, this very population remains at greatest risk for severe complications following viral infections, making vaccine prevention particularly relevant. MVA-BN was developed as a non-replicating smallpox vaccine that is potentially safer for people who are immunocompromised. In this phase II trial, 3 MVA-BN dosing regimens were evaluated for safety, tolerability, and immunogenicity in persons with HIV (PWH) who had a history of AIDS. Following randomization, 87 participants who were predominately male and African American received either 2 standard doses on weeks 0 and 4 in the standard dose (SD) group (N = 27), 2 double-standard doses on the same schedule in the double dose (DD) group (N = 29), or 3 standard doses on weeks 0, 4 and 12 in the booster dose (BD) group (N = 31). No safety concerns were identified, and injection site pain was the most commonly reported solicited adverse event (AE) in all groups (66.7%), with no meaningful differences between groups. The incidence of severe (Grade 3) AEs was low across groups and no serious AEs or AEs of special interest considered related to study vaccine were reported. Doubling the standard MVA-BN dose had no significant effect on induction of neutralizing antibodies, with 100% seroconversion and comparable GMTs at week 6 in the SD and DD groups (78.9 and 100.3, respectively). A booster dose significantly increased peak neutralizing titers in the BD group (GMT: 281.1), which remained elevated at 12 months (GMT: 45.3) compared to the SD (GMT: 6.2) and DD (GMT: 10.6) groups. However, based on the immune response previously reported for healthy participants, a third dose (booster) does not appear necessary, even for immunocompromised participants.

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1. Introduction

Despite eradication of smallpox, serious concerns persist about the re-emergence of variola virus as a global pathogen. These concerns have led to resumed recommendations for vaccination with traditional vaccinia-virus based smallpox vaccines, when medically indicated, in populations considered at risk of exposure to variola virus [1]. However, traditional replicating smallpox vaccines are associated with rare, but potentially serious adverse reactions, including progressive vaccinia, eczema vaccinatum, generalized vaccinia, post-vaccinial encephalitis, and myopericarditis [2–5]. Due to the possibility of viral replication post-vaccination, the risk of these adverse reactions is increased in immunocompromised populations, such as persons living with HIV (PWH) [6–10]. Moreover, immunocompromised individuals are also at higher risk for severe disease following viral infection [11], and therefore have an even greater need than the general population for protection in the event of exposure.

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The non-replicating modified vaccinia Ankara vaccine (MVA-BN) has been developed to address these safety concerns and has been shown to be substantially less reactogenic than traditional replicating smallpox vaccines [12,13]. To date, MVA-BN has been administered in over 20 clinical trials to approximately 8000 individuals, including those with HIV and atopic dermatitis. In the healthy adult population, MVA-BN is well tolerated [14–21] and induces immune responses comparable to traditional smallpox vaccines [12,13,22]. Similarly, in immunocompromised populations, no safety concerns with MVA-BN have been identified, including hematopoietic stem cell transplant (HSCT) recipients [23] and PWH who have CD4 cell counts of ≥200 cells/μL [24,25]. Based on these data, MVA-BN is currently approved for prevention of smallpox in Europe (trade name IMVANEX) and Canada (trade name IMVAMUNE), and in the United States for prevention of smallpox and monkeypox (trade name JYNNEOS). In addition to this approved MVA-BN smallpox and monkeypox vaccine, which is administered subcutaneously, there are several ongoing clinical programs examining MVA-BN-based recombinant vaccines using intramuscular injections (e.g., MVA-BN-RSV, MVA-BN-WEV, and MVA-BN-Filo). Clinical data suggest that both routes of administration are generally safe and immunogenic [12].

However, immune responses are attenuated following vaccination with a variety of licensed vaccines in PWH compared with the general population [26]. Aligned with this, a previous MVA-BN study found that antibody titers were significantly lower in PWH compared with healthy volunteers even though the antibody titers in the PWH group were considered protective [25]. This trial was therefore conducted to investigate whether two alternative vaccination strategies with MVA-BN, a double dose or a booster dose, could induce better immune responses without compromising the safety profile of MVA-BN. This trial also evaluated the safety and immunogenicity of MVA-BN in the most severely immunocompromised population to date, those with baseline median CD4 cell counts across treatment groups of <350 cells/μL. In fact, all participants had documented CD4 cell count nadirs of <200 cells/μL prior to vaccine administration, which corresponds to a history of stage 3 HIV, commonly known as acquired immunodeficiency syndrome (AIDS).

2. Materials and methods

2.1. Trial design

Following approval by the relevant institutional review boards, this randomized, open-label, Phase II parallel design trial was conducted at 12 sites in the U.S. between 2014 and 2017.

The primary endpoint was the occurrence, relationship, and intensity of any serious and/or unexpected adverse events during the trial. Immunogenicity endpoints included total serum and neutralizing antibodies measured by enzyme-linked immunosorbent assay (ELISA) and plaque reduction neutralization test (PRNT), respectively. Based on the results of these assays, geometric mean titers (GMTs) and seroconversion rates after vaccination with MVA-BN were calculated.

Enrollment of 90 HIV positive adults meeting the inclusion criteria was planned, with a 1:1:1 randomization scheme across 3 treatment groups. The standard dose (SD) group received 1 standard dose of MVA-BN on weeks 0 and 4 (the standard regimen); the double dose (DD) group received 2 standard doses of MVA-BN on weeks 0 and 4 (a double-dose regimen); and the booster dose (BD) group received 1 standard dose of MVA-BN on weeks 0 and 4 followed by a booster vaccination with 1 standard dose of MVA-BN on week 12 (a booster dose regimen). All doses of MVA-BN were administered subcutaneously with a 24 or 25 gauge needle in the upper arm according to standard clinical practice.

2.2. Participants

All study-related procedures were in accordance with the provisions of the Declaration of Helsinki (2013). Informed consent was obtained after participants had received full information about the study and possible adverse drug reactions.

Men and non-pregnant women between 18 and 45 years of age were eligible if they were vaccinia-naïve, had CD4 screening counts between 100 and 500 cells/μL, had plasma HIV-1 RNA screening results of <200 copies/mL, had a documented CD4 cell nadir of <200 cells/μL any time prior to screening, and were on stable antiretroviral therapy. Excluded from the trial were participants with typical vaccinia scars or a known history of smallpox vaccination; an uncontrolled serious infection; a history of or actively ongoing serious medical condition including autoimmune disease or malignancy; a history of coronary heart disease, myocardial infarction, angina pectoris, congestive heart failure, cardiomyopathy, stroke or transient ischemic attack, uncontrolled high blood pressure, or any other heart condition under the care of a doctor; an immediate family member with onset of ischemic heart disease before 50 years of age; or alcohol (≥40 g/day) or intravenous drug abuse within the past 6 months.

2.3. Vaccine

MVA-BN is a highly attenuated, purified live vaccine [27]. The MVA bulk drug substance was produced at Bavarian Nordic (Kvistgård, Denmark) according to cGMP standards, and filled, formulated and labeled at IDT Biologika GmbH (Dessau-Rosslau, Germany). The vaccine (batch number: F00102) was provided in liquid frozen 0.5 mL aliquots, and had a virus titer of $\geq 0.5 \times 10^8$ $TCID_{50}$ MVA-BN (standard dose). Each dose contains 0.61 mg Tris-hydroxymethyl-amino methane and 4.1 mg sodium chloride, with no preservatives or adjuvants. All participants received injections from the same batch, with the same viral titer, and those receiving a double dose received twice the number of injections on each administration day. The MVA-BN smallpox vaccine was shipped and stored at −4°F ± 9°F (−20 °C ± 5 °C), avoiding direct light. Vials were not to be re-frozen once thawed.

2.4. Safety assessments

Safety and reactogenicity included assessments of solicited local and systemic adverse events, unsolicited adverse events, and serious adverse events. Solicited adverse events constituted a set of pre-defined, expected local reactions (erythema, swelling, pruritus, induration and pain) as well as systemic reactions (elevated body temperature, headache, chills, myalgia, nausea and fatigue) listed on a memory aid provided to participants for an 8-day period following each vaccination. Unsolicited events reported by the participant or observed by the investigator were reported from week 0 to 8 for the SD and DD groups and from week 0 to 16 for the BD group, and consisted of any adverse event that was either not listed on the memory aid or occurred outside an 8-day post-vaccination solicitation period.

Safety laboratory tests were performed at screening, and 2 weeks after each vaccination; abnormal values assessed as being clinically significant by the investigator were documented as unsolicited adverse events. All unsolicited adverse events ongoing at week 8 (SD and DD groups) or week 16 (BD group) were followed until resolution, or at follow-up at week 56/64. The intensity of unsolicited and solicited adverse events was analyzed according to predefined grades, with Grade 3 considered a severe adverse event.

In this study, adverse events of special interest were defined as any: (1) cardiac symptoms, (2) clinically significant electrocardiogram (ECG) changes, or (3) increases in Troponin I that were ≥ 2

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times the upper limit of normal developing since the first vaccination. Participants developing an adverse event of special interest returned for a physical and cardiac examination and if indicated, further diagnostic tests. Follow-up of adverse events of special interest was continued until resolution or stabilization.

To monitor long-term safety, participants returned at 6 and 12 months after the final vaccination for follow-up visits.

2.5. Immunogenicity assessments

Serum antibody titers were measured by PRNT and ELISA on samples drawn at week 0 (baseline), week 4, week 6, and follow-up visits (week 30 and week 56 for the SD and DD groups; week 38 and week 64 for the BD group). For the BD group, additional samples were drawn at week 12 and week 14 due to the administration of the booster vaccine at week 12. The vaccinia-specific PRNT and ELISA utilized Western Reserve and MVA as antigens, respectively. These assays have been previously described [25] and were performed according to the most current modifications [19]. Immunogenicity assessments included antibody GMT and seroconversion rates.

2.6. Statistical methods

Statistical analyses were performed using SAS 9.4 (SAS-Institute, Cary, NC, USA).

A sample size of 30 participants in each group was planned to allow for detection of adverse events having an incidence of at least 1 in 10, with a detection probability of at least 95%. For the primary endpoint, all adverse events (solicited and unsolicited) with onset during the active trial phase (4 weeks following the final vaccination) and all serious adverse events with onset from first vaccination to 12 months after the final vaccination were analyzed.

For immunogenicity endpoints, the sample size provided approximately 80% power to detect a 4-fold difference between groups using the PRNT assay or a 2-fold difference using the ELISA assay in terms of GMT ratios, assuming a standard deviation (SD) of 0.85 for the $\log_{10}$ PRNT titers and an SD of 0.42 for the $\log_{10}$ ELISA titers. Immunogenicity comparisons were descriptive and not adjusted for multiple comparisons.

The PRNT GMT and ELISA GMT were calculated by taking the antilogarithm of the mean of the $\log_{10}$ titer transformations. Titers below the detection limit were assigned the value of 1. Seroconversion was defined as the appearance of antibody titers greater than or equal to the detection limit for initially seronegative participants and at least a doubling of the titer for initially seropositive participants.

Analyses of safety endpoints were based on the full analysis set comprising all randomized participants who received at least one vaccination. Analyses of immunogenicity endpoints were based on the per-protocol set, a subset of full analysis set participants

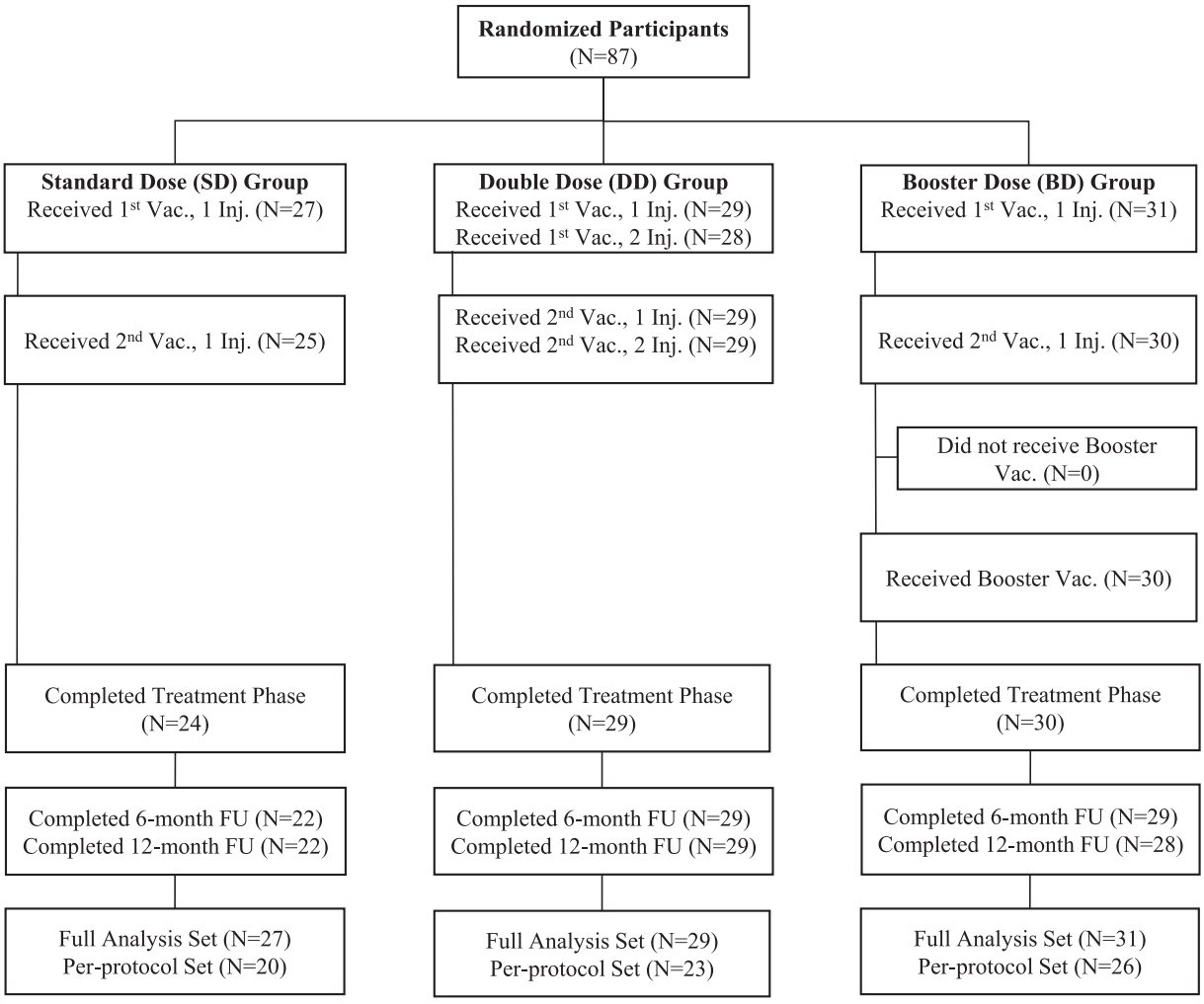

Fig. 1. Disposition of randomized participants. A total of 87 participants were randomized 1:1:1 to either the standard dose (SD), double dose (DD), or booster dose (BD) groups. Almost every participant received all vaccinations and completed the 6-month and 12-month follow-up visits.

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who received all vaccinations and adhered to all protocol conditions without major protocol deviations.

Randomization was conducted using an automated randomization system. The conduct of the trial was overseen by an independent data safety monitoring board.

3. Results

3.1. Clinical participant population and conduct of the study

A total of 87 participants were randomized, with 27 participants in the SD, 29 participants in the DD, and 31 participants in the BD groups, respectively (Fig. 1). All randomized participants received at least 1 vaccination, had data available for analysis, and were included in the safety analysis.

The first vaccination (at week 0) was received by all participants in all groups. However, 1 participant in the DD group only received 1 of the 2 scheduled MVA-BN injections on the first dosing day. The second vaccination (at week 4) was received by all participants except 2 in the SD group (1 was lost to follow up and 1 was incarcerated) and 1 participant in the BD group who withdrew from the study. The booster vaccination (at week 12) was received by all participants in the BD group who received the second vaccination.

The demographic and baseline disease characteristics across groups were comparable. Overall, the mean age of all participants was 35.0 years and the majority were male (86.2%) and African American (57.5%) (Table 1). All participants were on stable antiretroviral therapy, with 75.9% receiving at least one other concomitant medication. The most common other concomitant medications were psychoanaleptics (25.3%), antibacterials for systemic use (21.8%), and analgesics (18.4%). Less than half of all participants (40.2%) were classified as smokers. The median documented CD4 cell nadir for all participants prior to study participation was 102 cells/μL, with approximately 20% of all participants having a study baseline CD4 cell count of <200 cells/μL.

3.2. Overall safety assessment

No significant safety findings with MVA-BN were identified. While most participants experienced at least 1 adverse event (80.5%) during the active trial phase, 14.9% of participants experienced severe (Grade 3) adverse events and only 1 participant reported a serious adverse event (severe pancreatitis), or an adverse event of special interest (mildly increased troponin I; slightly above 2 times the upper limit of normal with no associated cardiac symptoms) (Table 2). The serious adverse event and adverse event of special interest were both reported in the BD group and considered unrelated to study vaccine by the investigator, occurring 31 days after the second (week 4) vaccination and 14 days after the booster (week 12) vaccination, respectively. A serious adverse event of life threatening or disabling depression was experienced by another participant in the BD group, outside of the active trial phase (177 days after the week 12 booster vaccination). No participant withdrew or discontinued from the study due to an adverse event and no deaths occurred during the trial.

When comparing adverse events across dose groups, no clinically meaningful differences were evident. Compared with the SD and DD groups, the BD group had a slightly higher incidence of overall adverse events (77.8% and 75.9% vs 87.1%, respectively) and related adverse events (74.1% and 72.4% vs 83.9%, respectively) (Table 2). These higher incidences observed in the BD group may be attributed to the additional time on trial required for administration of the booster vaccination. Severe adverse events (Grade 3) were reported by a similar small proportion (12.9% to 17.2%) of participants across all dose groups.

Although all solicited local events were deemed related to vaccine as specified in the study protocol, only 3 unsolicited adverse events were considered at least possibly related to the vaccine by the investigator and all were of mild (Grade 1) intensity. These events consisted of an episode of oropharyngeal pain following the second vaccination in the SD group, an elevated creatinine

Table 1
Demographic and Disease Characteristics of Study Participants at Baseline – Full Analysis Set.

Characteristic	SD Group (N = 27)	DD Group (N = 29)	BD Group (N = 31)	All Participants (N = 87)
Age, mean ± StD				
Years of age	35.1 ± 7.7	33.1 ± 6.7	36.6 ± 5.4	35.0 ± 6.7
Sex, n (%)				
Female	4 (14.8)	5 (17.2)	3 (9.7)	12 (13.8)
Male	23 (85.2)	24 (82.8)	28 (90.3)	75 (86.2)
Body Characteristics, mean ± StD				
BMI (kg/m^2)	26.1 ± 4.8	25.2 ± 3.6	26.2 ± 4.6	25.9 ± 4.3
Race, n (%)				
African American	16 (59.3)	18 (62.1)	16 (51.6)	50 (57.5)
Native Hawaiian/Other Pacific Islander	0	0	1 (3.2)	1 (1.1)
White/Caucasian	10 (37.0)	10 (34.5)	14 (45.2)	34 (39.1)
Other	1 (3.7)	1 (3.4)	0	2 (2.3)
Ethnicity, n (%)				
Hispanic or Latino	5 (18.5)	4 (13.8)	3 (9.7)	12 (13.8)
Not Hispanic or Latino	22 (81.5)	25 (86.2)	28 (90.3)	75 (86.2)
Time since HIV diagnosis, median (min - max)				
Years since diagnosis	6.6 (0–21)	6.5 (1–23)	5.8 (0–25)	6.4 (0–25)
CD4 T cell count (cells/μL), median (min - max)				
Nadir (prior to trial)	100 (10–200)	110 (0–199)	107 (2–391)[a]	102 (0–391)
Baseline Value	317 (142–469)	279 (145–479)	326 (104–500)	312 (104–500)
<200 cells/μL, n (%)	6 (22.2)	6 (20.7)	5 (16.1)	17 (19.5)
≥200 cells/μL, n (%)	21 (77.8)	23 (79.3)	26 (83.9)	70 (80.5)

BMI, body mass index; BD, booster dose; BMI, body mass index; DD, double dose; HIV, human immunodeficiency virus; SD, standard dose; StD, standard deviation; N, number of participants in the specified group; n, number of participants with data available; %, percentage based on N.
Note that weight and BMI were based on the weight at screening.
[a] One participant in the BD group had a documented CD4 cell nadir of 391 cells/μL but had a measurement of <200 cells/μL before study participation.

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value following the second vaccination in the DD group, and an event of myalgia following the booster vaccination in the BD group.

3.3. Solicited adverse events

The most commonly reported solicited local adverse event for all dose groups was injection site pain (66.7%) (Table 3), followed by injection site pruritis (43.7%), injection site erythema (42.5%), and injection site swelling (37.9%). Grade 3 adverse events were reported only for injection site pain (8.0%) and injection site pruritus (1.1%). When comparing across dose groups, the BD group showed a generally higher incidence of solicited local adverse events, consistent with the administration of an additional booster vaccination.

The most commonly reported solicited systemic adverse events for all participants were myalgia (39.1%), fatigue (35.6%), and headache (33.3%). Grade 3 events were most commonly reported for fatigue (5.7%). Grade 3 headache was only reported in the DD and BD groups (6.9% and 3.2%, respectively) and Grade 3 nausea was only reported in the DD group (6.9%).

For both local and systemic solicited adverse events, the median duration of events was 5 days or less across dose groups.

3.4. Unsolicited adverse events

Very few unsolicited adverse events were reported and the most commonly reported unsolicited adverse events for all participants by system organ class were infections and infestations (6.9%) and respiratory, thoracic, and mediastinal disorders (5.7%). In all participants, gastrointestinal disorders, investigations, and musculoskeletal and connective tissue disorders were experienced at the same low incidence (3.4%). All other adverse events by system organ class were experienced by<3% of participants. No unsolicited adverse events by preferred term were experienced by more than 1 participant in any dose group.

3.5. Immunogenicity

Nearly all participants were seronegative at baseline as measured by PRNT (98.6%) and ELISA (91.3%). MVA-BN induced a very similar humoral response up to week 6 following the first 2 vaccinations in all three groups (Fig. 2 and Table 4). At this timepoint, all participants had seroconverted except for 1 participant in the BD group by PRNT and neutralizing GMTs were highly comparable across groups (78.9, 100.3 and 95.9 in the SD, DD and BD groups respectively) even though the DD group had received a higher dose (Table 4). Despite a two-fold difference in dose, seroconversion rates remained comparable between the DD (72.7%) and SD (66.7%) groups after 12 months with comparable neutralizing GMTs (10.6 and 6.2, respectively; ratio: 1.695). Two weeks after the booster vaccination (week 14) all participants had seroconverted and the peak neutralizing GMT was more than 3-fold higher compared with the SD group (281.1 vs 78.9, respectively; ratio:

Table 2
Adverse Events by Dose Group During the Active Trial Phase – Full Analysis Set.

Parameter n (%)	SD Group (N = 27)	DD Group (N = 29)	BD Group (N = 31)	All Participants (N = 87)
At least one:				
Adverse event (solicited and unsolicited)	21 (77.8)	22 (75.9)	27 (87.1)	70 (80.5)
Unsolicited adverse event	5 (18.5)	6 (20.7)	10 (32.3)	21 (24.1)
Severe adverse event (Grade 3)	4 (14.8)	5 (17.2)	4 (12.9)	13 (14.9)
Serious adverse event[a]	0	0	1 (3.2)	1 (1.1)
Adverse events of special interest[a]	0	0	1 (3.2)	1 (1.1)
Related[b] adverse event	20 (74.1)	21 (72.4)	26 (83.9)	67 (77.0)
Related[b] severe adverse event (Grade 3)	4 (14.8)	5 (17.2)	3 (9.7)	12 (13.8)

BD, booster dose; DD, double dose; SD, standard dose.
[a] Serious adverse events and adverse events of special interest were included as occurring during the active trial phase even if the events continued beyond the 29-day follow-up period after each vaccination.
[b] An adverse event the investigator considered to have possible, probable, definite, or missing relationship to trial vaccine.

Table 3
Solicited Adverse Events by Local and Systemic Classification – Full Analysis Set.

Preferred Term n (%)	SD Group (N = 27)	DD Group (N = 29)	BD Group (N = 31)	All Participants (N = 87)
Local				
Injection site pain	17 (63.0)	19 (65.5)	22 (71.0)	58 (66.7)
Grade 3	2 (7.4)	4 (13.8)	1 (3.2)	7 (8.0)
Injection site pruritus	12 (44.4)	8 (27.6)	18 (58.1)	38 (43.7)
Grade 3	1 (3.7)	0	0	1 (1.1)
Injection site erythema	8 (29.6)	11 (37.9)	18 (58.1)	37 (42.5)
Injection site swelling	10 (37.0)	10 (34.5)	13 (41.9)	33 (37.9)
Injection site induration	8 (29.6)	6 (20.7)	10 (32.3)	24 (27.6)
Systemic				
Myalgia	9 (33.3)	12 (41.4)	13 (41.9)	34 (39.1)
Grade 3	1 (3.7)	0	1 (3.2)	2 (2.3)
Fatigue	9 (33.3)	11 (37.9)	11 (35.5)	31 (35.6)
Grade 3	2 (7.4)	2 (6.9)	1 (3.2)	5 (5.7)
Headache	9 (33.3)	10 (34.5)	10 (32.3)	29 (33.3)
Grade 3	0	2 (6.9)	1 (3.2)	3 (3.4)
Chills	7 (25.9)	6 (20.7)	6 (19.4)	19 (21.8)
Grade 3	1 (3.7)	0	0	1 (1.1)
Nausea	6 (22.2)	6 (20.7)	6 (19.4)	18 (20.7)
Grade 3	0	2 (6.9)	0	2 (2.3)
Elevated body temperature	1 (3.7)	0	0	1 (1.1)

BD, booster dose; DD, double dose; SD, standard dose. The incidence of Grade 3 events is only reported for preferred terms experienced by at least 1 participant.

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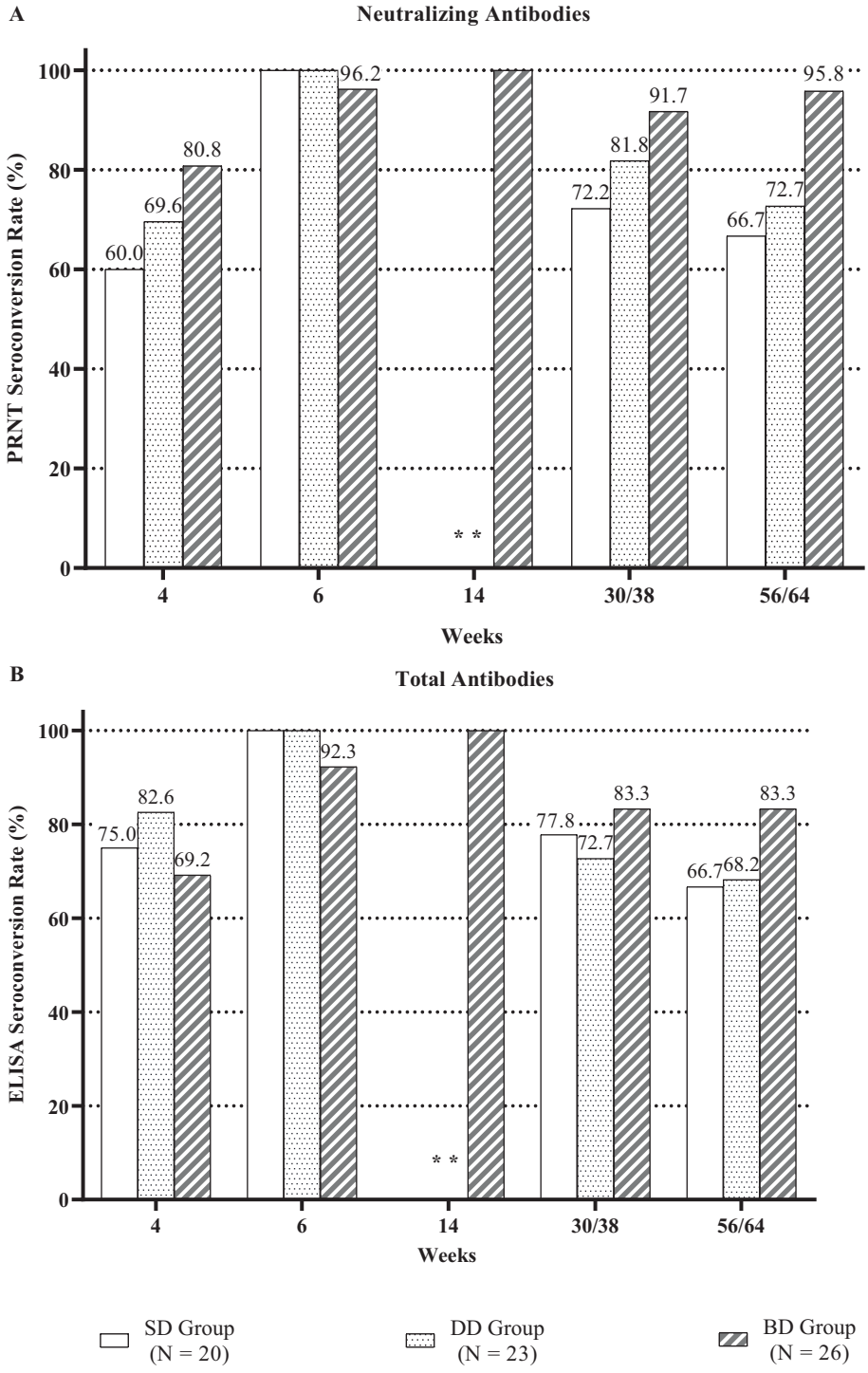

Fig. 2. Seroconversion rates following MVA-BN vaccinations. Neutralizing and total antibody responses were analyzed for the per-protocol set by plaque reduction neutralization test (PRNT) (A), and enzyme-linked immunosorbent assay (ELISA) (B), respectively.
** Note that week 14 is only an applicable timepoint for the booster dose (BD) group and corresponds to 2 weeks following the booster (i.e., final) vaccination. For the standard dose (SD) and double dose (DD) groups, week 6 corresponds to 2 weeks following the final vaccination. Month 6 were Week 30 for the SD and DD groups, Week 38 for the BD group; month 12 were Week 56 for the SD and DD groups, Week 64 for the BD group.

3.561). The week 12 booster vaccination in the BD group resulted in higher seroconversion rates and neutralizing GMTs compared to the SD group at 6 months (41.5 vs 6.2, respectively; ratio: 6.727) and 12 months (45.3 vs 6.2, respectively; ratio: 7.275).

Although GMTs detected by ELISA were generally higher in each group at each timepoint, the ratios between groups were similar to those obtained with PRNT (Table 4).

4. Discussion

No safety issues with MVA-BN smallpox vaccine were identified in immunocompromised individuals with a history of AIDS when administered as a standard 2-dose regimen alone, in combination with a booster, or when the standard dose was doubled. The majority of adverse events were transient local and systemic reactogenic

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Table 4
PRNT and ELISA GMTs and Group Ratios Over Time – Per-Protocol Set.

Assay Timepoint	GMT			GMT Ratio [95% CI]	
	SD Group (N = 20)	DD Group (N = 23)	BD Group (N = 26)	DD/SD Group	BD/SD Group
PRNT					
Week 0	1.0	1.0	1.2	–	–
Week 4	3.9	4.8	10.1	1.215 [0.491, 3.006]	–
Week 6	78.9	100.3	95.9	1.271 [0.663, 2.438]	–
Week 14			281.1	–	3.561 [1.846, 6.870] [a]
Month 6	6.2	11.5	41.5	1.868 [0.651, 5.359]	6.727 [2.493, 18.150]
Month 12	6.2	10.6	45.3	1.695 [0.583, 4.930]	7.275 [2.693, 19.649]
ELISA					
Week 0	1.5	1.2	1.8	–	–
Week 4	40.0	47.1	41.8	1.176 [0.357, 3.870]	–
Week 6	552.2	846.1	726.1	1.532 [0.750, 3.131]	–
Week 14			1591.2	–	2.882 [1.659, 5.006] [a]
Month 6	34.6	30.8	143.3	0.888 [0.230, 3.434]	4.138 [1.241, 13.793]
Month 12	25.2	27.5	116.2	1.091 [0.262, 4.548]	4.608 [1.365, 15.558]

BD, booster dose; CI, confidence interval; DD, double dose; ELISA, enzyme-linked immunosorbent assay; GMT, geometric mean titer; PRNT, plaque reduction neutralization test; SD, standard dose.

[a] GMT values from the SD group at week 6 and BD group at week 14 were used to calculate the SD/BD GMT ratio, corresponding to 2 weeks following the last vaccination in both dose groups.

events of mild to moderate intensity typically associated with modern injectable vaccines. Doubling the dose at the standard schedule did not increase the incidence of overall adverse events or severe adverse events (Grade 3). This finding is consistent with the similar safety profiles observed when comparing a single high dose of MVA-BN to the standard two-dose regimen in healthy adult volunteers [15]. Adding a booster vaccination following the standard regimen increased the overall incidence of adverse events during the active trial phase, which is expected since this group remained in the trial longer than the other study groups. However, serious adverse events were uncommon and experienced at similar rates in all 3 dose groups and the median duration of all solicited adverse events was 5 days or less. The comparable safety profile of MVA-BN observed, despite doubling the dose or adding a booster vaccination, likely reflects the highly attenuated nature of MVA-BN. The inability of MVA-BN to replicate in human cells [27] appears to allow for the safe administration of higher and more frequent doses, even in an immunocompromised population.

Cardiac events have been intensively monitored throughout the MVA-BN clinical development program, since reports of myopericarditis were reported in military personnel administered replicating smallpox vaccines [2,5,28,29]. Although one participant in the BD group had an episode of mildly increased troponin I slightly more than 2 times the upper limit of normal, this was not considered related to MVA-BN and was not associated with any cardiac symptoms. Therefore, the findings from this study support the observations of other MVA-BN clinical studies in healthy adults 18 to 80 years of age that have also not identified any cardiac safety concerns or reported any confirmed adverse events indicative of myo- or pericarditis [16,18,19].

In this immunocompromised population, the nature, number, and severity of both local and systemic reactogenicity symptoms were comparable to those reported in healthy adult volunteers [12–21] and less immunocompromised PWH [24,25] following MVA-BN administration. This acceptable safety profile is important because the pool of individuals at risk of complications following vaccination with replicating smallpox vaccines may be larger today compared to times when smallpox vaccinations were routinely performed [30]. In this context, vaccine-mediated protective immunity is of particular importance in immunocompromised individuals who are at higher risk for developing severe disease, if infected.

Doubling the dose of MVA-BN had a negligible effect on the kinetics and magnitude of the humoral response compared to the standard 2 dose regimen, similar to a previous report investigating a single high dose of MVA-BN in healthy adults [15]. In contrast, a third vaccination at week 12 significantly boosted the humoral response, which remained more durable than the standard 2-dose regimen. The ability to stimulate higher neutralizing antibodies through a booster vaccination has also been reported for traditional smallpox vaccines in vaccinia-experienced individuals and is presumably the result of antibodies with higher affinity induced after repeated antigen exposure [31].

While neutralizing antibodies are considered a correlate of protection against smallpox [32], a specific titer that is protective against variola has not been identified. However, when comparing the immune response observed in this study to another previously reported Phase III study conducted during an overlapping timeframe using the same laboratory testing procedures [13], immunocompromised PWH receiving MVA-BN appear to have a comparable immune response to healthy individuals receiving a traditional replicating vaccine (ACAM2000). In fact the peak neutralizing antibody response induced by MVA-BN was not only noninferior to ACAM2000 in the previous study, but the immune response induced by ACAM2000 was also within the same range as that observed in this study following 2 standard doses of MVA-BN (GMTs: 79.3 for ACAM2000 vs 78.9 in the SD group and 95.9 in the BD group) [13]. Since peak neutralizing antibody responses following the standard MVA-BN dose in PWH were comparable to those observed following ACAM2000 in healthy individuals, this study suggests that the immune response to the standard MVA-BN dose in individuals with a history of AIDS (i.e., CD4 cell nadir of <200 cells/μL) is likely protective. Although a booster dose was well tolerated and induced a higher and more durable antibody response in PWH, it does not seem necessary for smallpox protection.

This is the first study to administer MVA-BN in PWH who have a history of AIDS, using both standard and increased dose regimens. While the study suggests the standard regimen provides sufficient protection for this population, a double dose or an additional booster dose appear to have the same favorable safety profile.

5. Conclusion

This trial confirmed the established standard regimen of MVA-BN in terms of safety, tolerability and immunogenicity for use in immunocompromised, vaccinia-naïve individuals with a history

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of AIDS in whom replicating smallpox vaccines are contraindicated. Although a third booster dose produces a higher, more durable antibody response, the standard dose regimen of MVA-BN induces an immune response comparable to that historically associated with smallpox protection [13].

CRediT authorship contribution statement

Edgar Turner Overton: Supervision, Investigation, Writing - review & editing. **Steven J. Lawrence:** Supervision, Investigation, Writing - review & editing. **Jack T. Stapleton:** Supervision, Investigation, Writing - review & editing. **Heinz Weidenthaler:** Conceptualization, Methodology, Supervision, Writing - original draft. **Darja Schmidt:** Supervision, Investigation, Validation, Writing - original draft. **Brigitte Koenen:** Investigation. **Günter Silbernagl:** Formal analysis, Validation, Software, Writing - original draft. **Katrin Nopora:** Project administration, Writing - review & editing. **Paul Chaplin:** Conceptualization, Methodology, Supervision, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors Heinz Weidenthaler, Darja Schmidt, Brigitte Koenen, Günter Silbernagl, and Katrin Nopora are employees and stakeholders of Bavarian Nordic GmbH; Paul Chaplin is the company CEO. Drs. Edgar Turner Overton, Steven J. Lawrence, and Jack T. Stapleton were investigators for this study, which was funded by Bavarian Nordic. All authors attest they meet the ICMJE criteria for authorship.

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