



U.S. FOOD & DRUG
ADMINISTRATION

CMC Review Memorandum

DATE: July 22, 2019

TO: STN 125678/0

FROM: Afolabi C. Meseda, Ph.D., DVP, Animal Pharmacology Reviewer

THROUGH: Jerry P. Weir, Ph.D., Director, DVP
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APPLICANT: Bavarian Nordic A/S

STN: BL 125678/0

PRODUCT: MVA-BN, Smallpox (Modified Vaccinia Ankara) Vaccine, Live, Non-replicating

PROPOSED INDICATION: Active Immunization Against Smallpox and Monkeypox in Adults aged 18 Years and older

ACTION DUE DATE: September 24, 2019

RECOMMENDATION: Approval

ACTION TO BE FOLLOWED: Post-marketing assessment of clinical efficacy of MVA-BN against monkeypox (when feasible)

PRE-CLINICAL PHARMACOLOGY

Summary

In the absence of clinical smallpox, the evaluation of clinical efficacy of smallpox vaccines presents a special challenge. Thus, the evaluation of efficacy of new-generation smallpox vaccines like the candidate MVA-BN smallpox vaccine relies on the use of surrogate animal models. Although human monkeypox, a disease caused by monkeypox virus (MPXV) with clinical presentations similar to smallpox, is endemic in central Africa, where sporadic clinical outbreaks have been documented since the early 1970s, clinical trials to evaluate new-generation smallpox vaccines for the prevention of clinical monkeypox would be challenging, in part for logistic reasons.

Over the course of the development program for MVA-BN (also named IMVAMUNE under IND 11596 and in the preclinical studies described below), Bavarian Nordic A/S (Kvistgård, DENMARK) (BN; applicant) conducted a series of preclinical studies in animals (mice, rabbits, and non-human primates) that were designed to characterize the pharmacology and protective efficacy of the MVA-BN drug product (DP). Many developmental studies as well as immunogenicity and efficacy studies were conducted in (b) (4) mice, or (b) (4) mice (both immunocompetent and immunodeficient), and in non-human primate (NHP) models. Under IND 11596, reports on studies conducted in mouse models were reviewed by Dr. Alonzo Garcia (CMC reviewer for IND 11596 and STN BL 125678/0), who also presented high-level summaries of key studies conducted in mice in this original submission. This section of the review of the original STN 125678/0, and Amendments thereof, covers studies conducted in NHP models with the aim of determining whether data obtained in the NHP studies, when coupled with immunogenicity data obtained in pivotal clinical trials, would support an indication for immunization for the prevention of monkeypox in humans for the candidate MVA-BN smallpox vaccine.

Prior to the evaluation of MVA-BN in NHP models, the Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health (DMID/NIAID/NIH) funded a series of studies (under Master File (MF) (b) (4)) that were aimed at characterizing and developing orthopoxvirus challenge models that could be used in facilitating the evaluation of the protective efficacy of MVA-BN in NHP models. For this purpose, the DMID/NIAID/NIH engaged contract research organizations (CROs), including Battelle Biomedical Research Center, West Jefferson, Ohio (BBRC; Battelle), Southern Research Institute, Frederick, Maryland (SRI), Lovelace Biomedical and Environmental Research Institute, Albuquerque, New Mexico (LBERI), and the Health Protection Agency, Porton Down, UNITED KINGDOM (HPA) to study the course of monkeypox virus (MPXV) pathogenesis, morbidity and mortality, upon infecting cynomolgus macaques (*Macaca fascicularis*) via different challenge routes, including intravenous, intranasal, and intratracheal routes, as well as via respiratory aerosol challenge. The MPXV ((b) (4)) used in these studies as well as in the studies submitted in STN BL 125678/0 was originally isolated from the scab of a human case of monkeypox and was prepared as a clarified and concentrated cell lysate/supernatant of African green monkey (*Cercopithecus aethiops*) kidney cells (MA-104 Clone 1) at the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia. Lots of the MPXV animal challenge stock used in the studies below were obtained from the (b) (4)

Final reports on the DMID/NIAID/NIH-funded studies were previously

submitted to MF (b) (4). Data from these studies indicated that there is no single model that can recapitulate the course of smallpox disease in humans. The outcomes of these studies, including the determination of optimal challenge doses and defining the course of MPXV pathogenesis for the different challenge routes partly informed the design of studies evaluating the immunogenicity and protective efficacy of MVA-BN in different MPXV challenge models. Although the NHP models were not validated – validation would be impracticable – these studies were conducted in compliance with Good Laboratory Practices (GLP) per 21 CFR 58, using appropriate standard operating procedures (SOPs) developed by the CROs. Testing of antisera from the various preclinical studies for binding antibodies (IgG) by enzyme-linked immunosorbent assay (ELISA) and neutralizing antibodies by the plaque reduction neutralization test (PRNT) were performed by Bavarian Nordic at their (b) (4) facility per BN SOP/PRE/004 and SOP/PRE/030, respectively. The Modified Vaccinia virus Ankara (MVA) was used as the antigen in ELISA and the Western Reserve strain of vaccinia virus (VV-WR) was used as the target virus in the PRNT. Both ELISA and PRNT were developed and validated by the applicant. Assay validation reports have previously been submitted to IND 11596 and were reviewed by Dr. Alonzo Garcia.

Reviewer's Perspectives:

Although sporadic outbreaks of monkeypox often occur in central Africa, evaluation of the clinical efficacy of smallpox vaccines against monkeypox still presents a major challenge. Thus, the demonstration of clinical efficacy of smallpox vaccines against monkeypox, as is also the case with smallpox, relies on animal challenge studies. The MPXV/NHP model appears to be the most relevant in testing the efficacy of smallpox vaccines against monkeypox. Three main MPXV/challenge models (intravenous, intratracheal and aerosol) have been developed and characterized for this purpose. Although none of the three challenge models appears to sufficiently recapitulate the course of clinical monkeypox pathogenesis, nonetheless each model can be used as a surrogate for efficacy evaluation. The applicant conducted studies in which MVA-BN immunogenicity, and efficacy against MPXV, challenge in cynomolgus macaques were assessed using all three challenge models. The totality of the data from these studies indicates that MVA-BN induced vaccinia-specific binding and neutralizing antibodies in macaques, and vaccinated animals were protected from severe monkeypox morbidity and mortality upon subsequent challenge with MPXV by the intravenous and intratracheal routes, or by exposure to aerosolized MPXV. In each of four NHP studies in which a control arm of animals vaccinated with the US FDA-licensed ACAM2000 smallpox vaccine was included, MVA-BN was not as effective as ACAM2000 in preventing MPXV morbidity but elicited protective immune responses and enhanced survival by reducing morbidity when compared to placebo-treated macaques. Data from studies conducted in mouse models, including mousepox models using ectromelia virus challenge, also indicated that MVA-BN induced cross-protection against other orthopoxviruses (VV-WR and ectromelia virus), and could reasonably be expected to induce cross-protection against monkeypox in humans. Therefore, MVA-BN may be indicated for vaccination for the prevention of monkeypox. Continued evaluation of the efficacy of MVA-BN during any future deployment of MVA-BN as a countermeasure in instances of clinical monkeypox outbreaks is recommended post-licensure.

PHARMACOLOGICAL STUDIES OF MVA-BN IN NHP MODELS

Monkeypox

Monkeypox is a pustular disease caused by the monkeypox virus, a member of the genus orthopoxvirus in the *Poxviridae* family. The MPXV genome sequence and organization is similar to other orthopoxviruses, including vaccinia virus, cowpox virus, and variola virus. First discovered in 1958 in a colony of macaques, the first clinical case of monkeypox was documented in the Democratic Republic of the Congo (DRC) in 1970. Since then, sporadic outbreaks of monkeypox have been reported primarily in the DRC where monkeypox appears to be endemic, as well as in a few neighboring African countries, including Cameroon, Gabon, Central African Republic, Nigeria, and Cote d'Ivoire (source: CDC). From year 2003, however, clinical cases of monkeypox have been recorded in countries like the Republic of Congo, Israel, the United Kingdom, and the United States (US). An inadvertent introduction of MPXV into the US in 2003 resulted in 47 clinical cases of monkeypox in five Midwestern states (source: CDC). A monkeypox outbreak that began in Nigeria in 2017 has caused over 300 clinical cases and has resulted in exported cases reported in Singapore, Israel, and the United Kingdom. The transmission of MPXV parallels that of smallpox, and both animal-to-human transmission and human-to-human transmission are possible. Monkeypox is similar to smallpox in clinical presentation and disease progression, except that lymphadenopathy is associated with monkeypox and mortality rate (1 to 10%) is relatively lower than the ~ 30% mortality rate associated with smallpox caused by variola major.

The primary basis for the use of vaccinia virus strains as smallpox vaccines lies in the ability of orthopoxviruses to induce cross-protecting immune responses. Currently, there are no monkeypox-specific countermeasures, but the same therapeutic (antivirals and vaccinia immune globulin) and prophylactic (smallpox vaccines) countermeasures used for smallpox are also recommended for monkeypox by the CDC.

The published genomic DNA sequence of MVA-BN shows it contains 165041 base pairs (bp) (*GenBank* Accession # DQ983238), while the chorioallantois vaccinia Ankara (CVA), the replication-competent parent strain from which MVA was derived, contains 192353 bp (*GenBank* Accession # AM501482), a difference of 27312 bp, representing a 14.2% genome deletion in MVA-BN. ACAM2000 has a genome of 199234 bp (*GenBank* # AY313847), MPXV (Zaire strain 1975-005) a size of 196959 (*GenBank* # HM172544), variola major (strain Bangladesh-1975) a size of 186103 (*GenBank* # L22579), and variola minor a size of 186986 (*GenBank* # Y16780). Thus, the genome size of MVA-BN, like other strains of MVA, is smaller than the genome size of variola virus, MPXV and replication-competent vaccinia virus strains. Most of the genes deleted in MVA are host-range and immunomodulatory genes, and several studies have shown that major vaccinia virus antigens that induce protective immune responses against orthopoxviruses, including neutralizing antibodies, in animal models are present and expressed by MVA. These antigens include L1, A27, D8 and H3 of the matured virion form and A33 and B5 of the enveloped virion form of vaccinia virus.

Evaluation of MVA-BN Against MPXV in Cynomolgus Macaques

In evaluating the immunogenicity and protective efficacy of the candidate MVA-BN smallpox vaccine in NHP models, a total of eleven preclinical studies were conducted in NHP/MPXV challenge studies. All studies were conducted in cynomolgus macaques. Cynomolgus macaques are one of the best characterized models for susceptibility to MPXV infection, and one in which the clinical course of MPXV pathogenesis is similar to the clinical course of variola virus pathogenesis in humans.

Following the review of the applicant's meeting information package for a Type C pre-BLA meeting request to discuss CMC issues in June 2017, CBER requested the applicant to include a total of six full study reports in which the immunogenicity and protective efficacy of MVA-BN were investigated in cynomolgus macaques. However, an additional NHP study report was included in STN 125678/0 after the pre-BLA meeting (meeting information package submitted in Amendment 360 to IND 11596). Thus, a total of seven immunogenicity/efficacy studies conducted in cynomolgus macaques/MPXV challenge models were submitted as full reports in the original STN BL 125678/0, and are as follows: BN-PRE-08-004, BN-PRE-12-003; BN-PRE-08-005; BN-PRE-09-003; BN-PRE-09-004; BN-PRE-11-021; and BN-PRE-12-028. These studies were contracted to CROs, with all studies containing a placebo treatment arm, and four studies containing a control arm of macaques vaccinated with ACAM2000 smallpox vaccine. All MVA-BN lots used in the NHP studies were clinical lots manufactured according to good manufacturing practices. Although this Biologics License Application (STN BL 125678/0) was submitted to seek marketing authorization for the liquid-frozen (LF) formulation of MVA-BN, (b) (4) formulation of the vaccine was also evaluated in three of the studies described below. Tris-buffered saline (TBS; diluent for MVA-BN, clinical lots) was used as the placebo in all studies. Lots of ACAM2000 vaccine used in studies containing ACAM2000 vaccination arms were sourced from the Strategic National Stockpile (SNS) of the CDC. The NHP study reports are reviewed in this section. All MVA-BN and TBS inoculations were administered subcutaneously (SC) as proposed for human vaccination in STN 125678/0 and ACAM2000 was inoculated percutaneously (PC) as licensed for human vaccination.

Overall, data from these studies indicated that the candidate MVA-BN smallpox vaccine, when inoculated into cynomolgus macaques at the dose and prime/boost schedule proposed for use in humans, induced high titers of vaccinia-specific binding antibodies as well as varying levels of vaccinia-neutralizing antibodies. Protection of MVA-BN vaccinated macaques in the different studies ranged from 83-100%. In the four studies with an ACAM2000 vaccination arm, macaques in the ACAM2000 group were 100% protected. Further, monkeypox morbidity was less severe in ACAM2000-vaccinated macaques than MVA-BN vaccinated macaques. MVA-BN was not as effective as ACAM2000 in protecting macaques against monkeypox morbidity and mortality in the intravenous and intratracheal challenge models. Nonetheless, macaques vaccinated with MVA-BN were better protected against monkeypox morbidity and mortality than unvaccinated macaques, suggesting that MVA-BN enhanced survival against monkeypox, and should be effective in protecting humans from monkeypox.

Study BN-PRE-08-004 (NHP/MPXV Intravenous Challenge)

Title

Comparison of the Immunogenicity and Protective Efficacy of IMVAMUNE® and ACAM2000™ in the Intravenous Monkeypox Challenge Model in Cynomolgus Monkeys

Study Design Summary

Study BN-PRE-08-004 was conducted under Study protocol # 12425.01a at SRI. This was a vaccine dose-ranging study designed to evaluate the immunogenicity and protective efficacy of MVA-BN smallpox vaccine in a MPXV intravenous challenge model in cynomolgus macaques by comparing monkeypox morbidity and mortality in macaques challenged with MPXV after

vaccination with ACAM2000 smallpox vaccine (Table 1; adapted from Tables 1 and 2 (page 15) of Study Report BN-PRE-08-004 in STN BL 125678/0).

Table 1. Design of Study BN-PRE-08-004

Group	No. of macaques	Male/Female	Vaccination	Dose	Route	Vaccination schedule (study day)	MPXV challenge on day 63 (PFU)
1	3	1/2	TBS	N/A	SC	0, 28	5×10^7
2	5	3/2	MVA-BN	1×10^6 TCID ₅₀	SC	0, 28	5×10^7
3	5	3/2	MVA-BN	1×10^7 TCID ₅₀	SC	0, 28	5×10^7
4	5	2/3	MVA-BN	1×10^8 TCID ₅₀	SC	0, 28	5×10^7
5	6	3/3	MVA-BN	1×10^8 TCID ₅₀	SC	28	5×10^7
6	6	3/3	ACAM2000	2.5- 12.5×10^5 PFU	PC	28	5×10^7

N/A, Not Applicable; SC, subcutaneous; PC, percutaneous.

Clinical-grade LF formulation of MVA-BN (lot # 0030707) and TBS lot # (b) (4) used in the study were supplied by Bavarian Nordic. ACAM2000 (lot # VV04-003-A) and ACAM2000 diluent (lot # (b) (4)) were obtained from the CDC. MPXV Lot (b) (4) was used as the challenge virus.

Thirty (30) specific pathogen-free cynomolgus macaques aged 2-8 years were sourced from (b) (4). After quarantine, animals were randomized into six groups. Three groups-of-five macaques were vaccinated with 1×10^6 , 1×10^7 or 1×10^8 TCID₅₀ of MVA-BN, respectively, on study Days 0 and 28. A group-of-six macaques received 1×10^8 TCID₅₀ of MVA-BN on Day 28, and another group-of-six received the human dose ($2.5-12.5 \times 10^5$ PFU) of ACAM2000 on Day 28. A group-of-three macaques received, TBS on Days 0 and 28. All MVA-BN and TBS inoculations were administered subcutaneously in the upper left leg and ACAM2000 was inoculated percutaneously in the interscapular region. Serum samples collected 14 days before vaccination, as well as on study Days 0, 7, 14, 21, 28, 35, 42, and 56, were tested for total binding antibodies (IgG) by ELISA and neutralizing antibodies by PRNT at Bavarian-Nordic. Although the study protocol indicated the collection of post-vaccination blood samples (Days 28 and 56) for antibody arrays and Day -14 and 56 blood samples for interferon-gamma (IFN- γ) by enzyme-linked immunospot (ELISPOT) assay, there were no data submitted on antibody arrays or IFN- γ response. It is unlikely however, that data on antibody array would have any significant impact on the assessment of vaccine immunogenicity. With respect to the IFN- γ assay, as a measure of cell-mediated immune response, the applicant provided some data in another study (see review of Study BN-Pre-12-003 below).

On Day 63, all macaques were anesthetized with ketamine (10-30 mg/kg) and challenged by intravenous infusion of 5×10^7 PFU of MPXV into the saphenous vein. After MPXV challenge,

blood samples for viral load determination by real-time quantitative polymerase chain reaction (qPCR) targeting the MPXV (b) (4) gene encoding hemagglutinin, were collected on Days 63, 69, 72, 75, 78, 81, 84, 87, and 91. Following an information request sent to the applicant, a qPCR assay validation report (report # HAVLA-08-04R-F) prepared by (b) (4) was submitted in Amendment 28 to STN 125678/0. The validation report indicates that the assay met acceptance criteria for performance with regard to specificity, precision, accuracy, repeatability, reproducibility and linearity, and was thus suitable for the quantitation of MPXV genome.

Following MPXV challenge, animals were evaluated at least twice daily for clinical illness, behavioral changes, inappetence and activity, and morbidity. Weight changes, body temperature changes and pock lesions were recorded every 3 days after challenge through the end of study and lesions were photographed. Macaques were evaluated and scored for: depression, weakness, unresponsiveness, dehydration, dyspnea, cough, inappetence, nasal discharge, and edema, based on severity of each condition. A clinical score of "0" indicate no clinical sign; a score of "1" is mild clinical sign; a score of "2" is moderate clinical sign; and a score of "3" is severe clinical sign. Euthanasia criteria included significant weight loss, numerous pock lesions, elevated body temperature, anorexia, inability to eat or drink, dehydration and lethargy. Euthanasia decision was taken after consultation between the Study Director and the veterinarian and was reached when medication-free clinical support for adequate relief from pain/distress could no longer be provided. Animals were euthanized by intravenous injection of sodium pentobarbital (65 mg/kg).

Immunogenicity Results

The initial study plan was for (b) (4) to perform immunogenicity assays on post-vaccination serum samples under a technology transfer agreement with the applicant. However, in Amendment 1 to Study BN-PRE-08-004, (b) (4) indicated that the agreement was terminated by the applicant, who then assessed immunogenicity by ELISA and PRNT. Data from the immunogenicity assays were included in Amendment 1 to the final study report on BN-PRE-08-004 and submitted in STN 125678/0.

All macaques vaccinated with ACAM2000 developed a pock lesion (vaccine take) that peaked on Day 35 (i.e., 7 days post-vaccination). Pock lesion sizes ranged from 0.022 to 0.716 cm² (mean = 0.31 ± 0.26 standard deviation (SD)), with two macaques developing small lesion sizes. No macaque in the MVA-BN cohort or TBS group developed a vaccine take at any timepoint.

With the exception of macaque # 4438, serum samples from the TBS group had no vaccinia-specific IgG at any timepoint. Macaque #4438 had an IgG titer ≥ 100 (range, 100 to 637) at all timepoints; with the highest titer of 637 detected in the Day-28 serum sample. Antibody data for this macaque was excluded from analysis on the assumption that the antibody detected was a false positive. However, this reviewer believes the detection of vaccinia-specific IgG in macaque # 4438 may be real (see "Results of MPXV Challenge" below). Among macaques in group 2, one of five (1/5) was seropositive on Day 14, and peak IgG response (GMT = 129 was recorded on Day 35 (80% seropositivity). Macaque #4443 in group 2 failed to seroconvert even after the inoculation of a booster dose. All macaques in groups 3 and 4 were seropositive from Day 14 (mean Day 14 IgG GMTs were 174 and 974, respectively). Peak IgG titers of 3178 and 18588 for groups 3 and 4, respectively, were recorded on Day 35 (Table 2; adapted

from Table 4 and Appendix 1 of Amendment 1 to study report BN-PRE-08-004 in STN BL 125678/0). Thus, for the three MVA-BN prime/boost treatment groups (groups 2 to 4) peak IgG titers were recorded one week after vaccine boost (i.e., study Day 35). A peak IgG titer of 1073 was recorded on Day 42 (i.e., 14 days post-vaccination) in the single-dose MVA-BN group (group 5), similar to the mean GMT for group 4 on Day 14. A mean peak GMT of 606 was also recorded for the ACAM2000 treatment group on Day 42 (i.e., 14 days post-vaccination). The relatively lower peak GMT for the ACAM2000 group compared to the same timepoint for 10^8 TCID₅₀ MVA-BN may in part be due to the higher dose of MVA-BN inoculated (i.e., 10^8 TCID₅₀ (~ 7×10^7 PFU) of MVA-BN) as well as the use of MVA as the coating antigen in ELISA. Whereas the total antibody response to ACAM2000 will include several host range and immunomodulatory gene products that are deleted in MVA, the immune response to MVA-BN will exclude the antigens absent in MVA. Thus, antibodies induced in response to ACAM2000 host range and immunomodulatory genes will not be detected in the applicant's ELISA as part of the total IgG response. This, plus the higher dose of MVA-BN, could also account in part for the 33 to 40% seropositivity (IgG GMT of 7) recorded seven days after vaccination with 10^8 TCID₅₀ of MVA-BN when macaques in the ACAM2000 group had no detectable IgG at the equivalent timepoint.

Table 2. Immunogenicity of MVA-BN Smallpox Vaccine in Study BN-PRE-08-004

Group	Vaccination (Schedule Day)	ELISA peak GMT (% seroconversion)	PRNT peak GMT (% seroconversion)
1	TBS (0, 28)	1 (0)	1 (0)
2	1×10^6 TCID ₅₀ (0, 28)	129 (80)	182 (100)
3	1×10^7 TCID ₅₀ (0, 28)	3178 (100)	620 (100)
4	1×10^8 TCID ₅₀ (0, 28)	18588 (100)	1518 (100)
5	1×10^8 TCID ₅₀ (28)	1073 (100)	243 (100)
6	$2.5\text{-}12.5 \times 10^5$ PFU ACAM2000 (28)	606 (100)	549 (100)

GMT, geometric mean titer

Neutralizing antibodies were not detected in macaques in group 1, including macaque # 4438. Two of five (2/5) macaques in group 2 had neutralizing antibodies (GMT = 3) on Day 14. Similarly, 60% of macaques in groups 3 and 4 had detectable neutralizing antibodies (GMT = 3 and 4, respectively) on Day 7. By Day 14, 5/5 (GMT = 56) and 5/5 (GMT = 90) of macaques in groups 3, and 4, respectively, had neutralizing antibodies. Mean peak neutralizing antibody titers of 182 (range, 27 to 529), 620 (range, 230 to 1251), and 1518 (range, 682 to 2743) were recorded on Day 35 for groups 2, 3, and 4, respectively (Table 2). The immunogenicity data for the MVA-BN prime/boost treatment groups indicate a linear relationship between the dose of MVA-BN and total IgG response, as well as between dose and neutralizing antibody titers. However, it seems counterintuitive that a higher peak GMT of neutralizing antibodies (PRNT titer) and seropositivity than total peak IgG GMT (ELISA titer) and seropositivity were obtained for group 2. PRNT titer for the single-dose MVA-BN group (group 5) was highest on Day 56 (GMT = 243). It is not clear if this was the peak for the group since no post-vaccination antisera were tested beyond Day 56. By contrast, the equivalent PRNT titer for group 4 at the same timepoint (i.e., 28 days post-vaccination) was 16. The applicant claimed that neutralizing antibodies were recorded at an earlier timepoint (Day 7) in macaques in groups 4 and 5 (GMT = 4 and 23; seropositivity = 60% and 83%, respectively) than the ACAM2000 treatment group (GMT = 2; seropositivity = 33%). While this claim is true, it is no indication that MVA-BN

protects better than ACAM2000 (see morbidity and mortality results below) considering the low antibody PRNT titers at the Day 7 timepoint. But nonetheless, immunogenicity data obtained in this study indicate that MVA-BN when administered in a prime/boost schedule as intended for clinical use induced high titers of binding and neutralizing antibodies.

Results of MPXV Challenge (MPXV Morbidity and Survival)

All macaques in the TBS group developed mild-to-moderate signs of depression and lethargy, moderate dehydration, moderate-to-severe anorexia, and edema. The three macaques became moribund and were euthanized on Days 74, 75, and 84. Macaque # 4438 that the applicant deemed to have yielded false-positive IgG titers was the last to succumb on Day 84, albeit with high viremia and pock lesions too numerous-to-count (TNTC), suggesting the likelihood that the animal might have had a low level of prior immunity. Signs of depression and lethargy were observed in 3/5 macaques in group 2, including macaque #4448 that had the highest peak PRNT titer of 529 on Day 35. Two of group 2 macaques were euthanized on Day 71, and macaque #4448 on Day 75. One of the two group 2 survivors had moderate edema. Three macaques in the single-dose MVA-BN group (group 5) and two macaques in group 3 had edema. All other vaccinated macaques survived (Table 3; adapted from Appendices 6 and 7 of Study Report BN-PRE-08-004 in STN 125678/0) with mild dehydration but no clinical signs of disease.

Table 3. MPXV Morbidity and Mortality in Study BN-PRE-08-004

Group	Vaccination (Schedule Day)	No. of Macaques	Peak Mean Total Body Lesion Count (Range)	^c Peak Mean Blood MPXV Load (genome copies/mL)	Survival (%)
1	TBS	3	700 (TNTC ^a)	1.3x10 ⁸	0/3 (0)
2	1x10 ⁶ TCID ₅₀ (0, 28)	5	629 (486- TNTC)	5.0x10 ⁷	2/5 (40)
3	1x10 ⁷ TCID ₅₀ (0, 28)	5	263 (25 to 538)	7.7x10 ⁵	5/5 (100)
4	1x10 ⁸ TCID ₅₀ (0, 28)	5	108 (5 to 316)	3.3 x10 ⁴	5/5 (100)
5	1x10 ⁸ TCID ₅₀ (28)	6	391 (42 to TNTC)	5.4x10 ⁶	6/6 (100)
6	2.5-12.5x10 ⁵ PFU ACAM2000 (28)	6	^b 4 (0 to 25)	^d < 5x10 ³	6/6 (100)

^aTNTC was arbitrarily assigned a value of 700 for calculation purposes.

^bOnly one macaque had a count of 25 at this timepoint and had no lesions at any other timepoint.

^cA viral load below the limit of detection (LOD) of 5x10³ genome copies/mL was assigned the LOD value for calculation.

^dAll macaques were below the limit of detection of 5x10³ genome copies/mL.

Body Weight and Body Temperature

Macaques in the TBS group experienced a decline in body weight from Day 66 until they succumbed to MPXV. Macaques in other treatment groups experienced transient body weight declines from Day 69 to 74, and regained weight thereafter, including the two group 2 survivors. In a similar pattern, macaques in the TBS group experienced a spike in body temperature on Day 66, which dropped below baseline at the time of euthanasia, particularly

for the macaque euthanized on Day 84 (body temperature was 14% below baseline). All surviving macaques experienced transient changes in body temperature.

Pock Lesions

All macaques that were euthanized due to disease severity (all three in group 1, and 3/5 in group 2) developed pock lesions that were TNTC before they were euthanized. Peak mean lesion counts were recorded on Day 72 (Table 3; and Figure 1, page 31 of Study Report BN-PRE-08-004 in STN BL 125678/0). Among macaques in the MVA-BN prime/boost cohort mean lesion counts were 629 (range, 486 to TNTC), 263 (range, 25 to 538), and 108 (range, 5 to 316) for groups 2, 3, and 4, respectively. The two survivors in group 2 had pock lesions through Day 84 (Day-84 mean = 68) and one had 3 lesions at the end of the study (Day 91). Similarly, all macaques in group 3 had pock lesions through Day 84 (Day-84 mean = 70), 3/5 had lesions on Day 87, and 1/5 had 6 lesions on Day 91. In group 4, all had lesions from Day 69 to 75. By Day 78, one macaque had cleared lesions while the remaining 4 had lesions (Day-78 mean = 40) and 3/5 still had lesions on Day 84 (Day-84 mean = 9), but all were lesion-free by Day 87.

In group 5, a peak mean lesion count of 391 (range, 58 to TNTC) was recorded on Day 72, with 50% of macaques in the group having lesions categorized as TNTC at this timepoint. Lesions on group 5 macaque # 4446 remained TNTC through Day 84, with a count of 75 on Day 91, and four of six (4/6) macaques in the group remained with pock lesions on Day 87 (Day 87 mean = 36). Except for macaque # 4442, which had 25 pock lesions on Day 72, all macaques in the ACAM2000 treatment group (group 6) were lesion-free throughout the observation period. In the applicant's analyses, a significant statistical difference was obtained in comparing the single-dose MVA-BN versus ACAM2000 groups ($P = 0.023014$; Tukey test). However, the applicant claimed that the difference in pock lesion count between

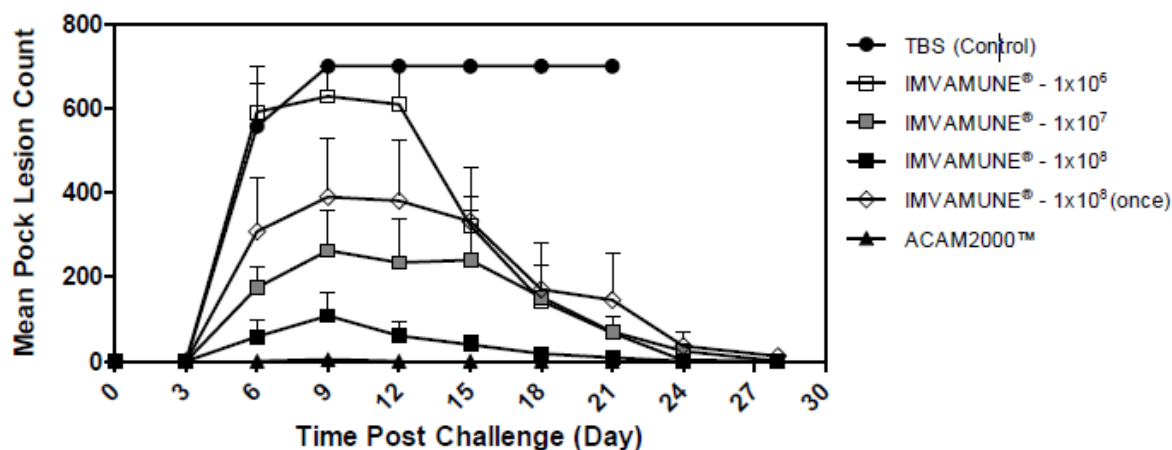


Figure 1. Mean Pock Lesions (\pm SEM) Post-MPXV Challenge in Study BN-PRE-08-004

the prime/boost 10^8 TCID₅₀ MVA-BN and ACAM2000 groups was not statistically significant ($P = 0.991021$; Tukey test). The single-dose 10^8 TCID₅₀ MVA-BN group was also not statistically different from the TBS group.

Thus, ACAM2000 was more effective than MVA-BN (both single-dose and prime/boost 10^8 TCID₅₀) in preventing pock lesion development based on the data submitted in this report.

Blood Viral Load

Generally, peak viremia was recorded between Days 69 to 75, except in ACAM2000-vaccinated macaques where MPXV genome was below the limit of detection (LOD) of 5000 genome copies/mL in all macaques, and at all timepoints. Peak blood viral load (viremia) in macaques in the TBS group was recorded on Days 72 to 75. Macaque # 4438 had a peak viral load of 7.30×10^7 genome copies/mL on Day 75, the other two macaques had peak viral loads of 1.12×10^8 and 2.64×10^8 genomes/mL on Day 74 and 75, respectively. Among the prime/boost MVA-BN treatment cohort, peak mean viral load appears to be directly proportional to vaccine dose. Thus, peak mean loads of 5.0×10^7 (Day 69), 7.7×10^5 (Day 72), and 3.3×10^4 (Day 69) genomes copies/mL were recorded for groups 2, 3, and 4, respectively (Table 3; and Figure 2, page 30 of Study Report BN-PRE-08-004 in STN BL 125678/0). With the exception of two macaques in group 4, all macaques in the prime/boost MVA-BN cohort experienced viremia at least once. All macaques in group 4 had viral load below the LOD from Day 75.

Macaques in group 5 (single-dose MVA-BN) experienced peak viremia on Day 72, with a mean viral load of 5×10^6 genome copies/mL at peak. All macaques in group 5 had detectable MPXV at least once after MPXV challenge but were all at below LOD levels by Day 78.

Thus, assessment of viral load after MPXV challenge in study BN-PRE-08-004 indicates a dose response to MVA-BN, with macaques vaccinated prime/boost with 10^8 TCID₅₀ MVA-BN being best protected but still less well protected from morbidity than ACAM2000-vaccinated macaques.

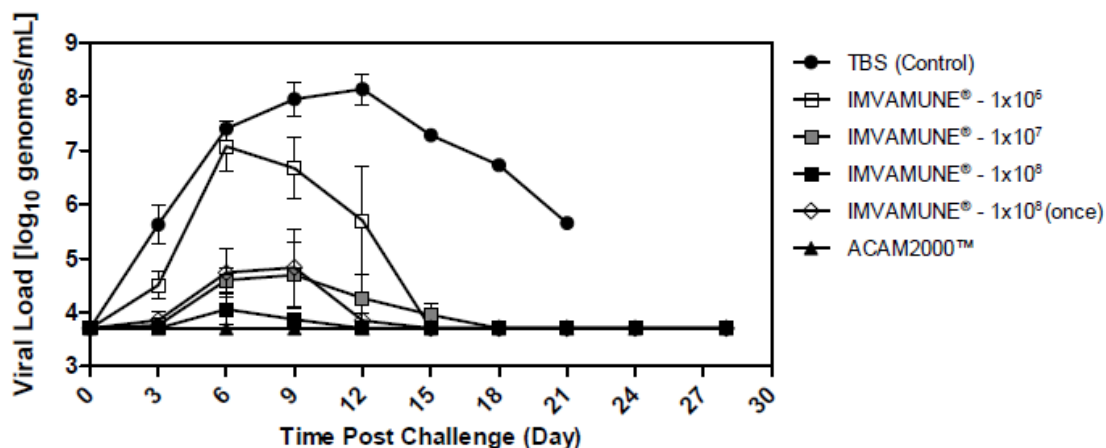


Figure 2. Mean Blood Viral Load Following MPXV Challenge in Study BN-PRE-08-004

Review comment: Data from study BN-PRE-08-004 indicated that MVA-BN administered prime/boost induced dose-dependent levels of binding and neutralizing antibodies in macaques, and protection of animals from intravenous MPXV challenge. The proposed clinical dose and schedule of MVA-BN vaccination conferred total protection on cynomolgus macaques and reduced monkeypox morbidity but was less effective than the clinical dose of ACAM2000 in reducing morbidity.

Study BN-PRE-12-003 (NHP/MPXV Intravenous Challenge)

Title

Comparison of the Efficacy and Immunogenicity of (b) (4) Liquid-frozen Formulations of IMVAMUNE® in the Intravenous Monkeypox Challenge Model in Cynomolgus Macaques.

Study Design Summary

Study BN-PRE-12-003 was conducted as Study No. 2594-100011378 at the BBRC, West Jefferson, Ohio. The study was designed to evaluate the immunogenicity and protective efficacy of the LF (b) (4) formulations of MVA-BN smallpox vaccine in a monkeypox intravenous challenge model in cynomolgus macaques. This study had no ACAM2000 arm. Clinical-grade MVA-BN LF formulation (lot # F00011), (b) (4), and TBS lot # (b) (4) used in the study were supplied by the applicant. Thirty-eight (19 male and 19 female) healthy, specific pathogen-free macaques weighing 2.6-5.5 kg and aged 3.7-5.2 years were sourced from (b) (4) for the study. Macaques were quarantined and acclimatized at BBRC and were individually housed under conducive environmental conditions of temperature, lighting and humidity. Eight days before the initiation of the study, macaques were weighed and randomized by age and sex into three treatment groups (Table 4; adapted from page 16 of Study Report BN-PRE-12-003 in STN BL 125678/0).

Table 4. Design of Study BN-Pre-12-003

Group	No. of macaques	Vaccination	Dose (TCID ₅₀)	Route	Vaccination schedule (study day)	MPXV challenge on day 63 (PFU)
1	10	TBS	N/A	SC	0, 28	5x10 ⁷
2	14	MVA-BN LF	1x10 ⁸	SC	0, 28	5x10 ⁷
3	14	MVA-BN (b) (4)	1x10 ⁸	SC	0, 28	5x10 ⁷

N/A, Not Applicable; SC, subcutaneous

MPXV lot (b) (4) was used as the challenge virus. A day before the study began, blood samples were drawn from macaques for immunogenicity assays (IgG ELISA and PRNT). On study Day 0, ten (10) macaques in the control group (group 1) were inoculated with 0.5 mL TBS and 14 macaques in each of groups 2 and 3 were inoculated with 1 x10⁸ TCID₅₀ of MVA-BN LF (b) (4), respectively. All inoculations were delivered via the subcutaneous route. Post-vaccination blood samples for ELISA and PRNT were collected on study Days 7, 14, 21, and 28. On study Day 28, all macaques received a second vaccination identical to the treatment on Day 0. Blood samples were collected on study Days 35, 42, and 56 for ELISA and PRNT. In addition, cell-mediated immune responses were assessed in a non-GLP compliant IFN-γ ELISPOT assay at (b) (4). For this purpose, peripheral blood mononuclear cells (PBMCs) obtained from macaques a day before vaccination (study Day -1) and on Day 56 after the first

vaccination were re-stimulated with VV-WR ((b) (4)) at a multiplicity of infection of 1.0, and tested for IFN- γ expression by a single-color ELISPOT assay following (b) (4) SOP.

On Day 56, macaques were transferred to a Biosafety Level 3 (BL-3) facility to acclimatize for MPXV challenge. On study Day 63, macaques were anesthetized by intramuscular injection of Telazol (3 mg/kg) and challenged with a target dose of 5×10^7 PFU of MPXV by intravenous injection into the left saphenous vein. Blood samples and throat swabs for the assessment of viremia and virus shedding were collected on the day of challenge and every 3 days through day 87 (i.e., study Days 66, 69, 72, 75, 78, 81, 84, and 87) and Day 91 or 92. Monkeypox lesions were photographed in parallel and lesions on the arms, legs, ventral torso, head and dorsal torso were counted. Macaques were also weighed on these days (final weighing on Day 91).

Macaques were monitored at least twice daily for behavior, physical appearance, feces output, eating behavior, and movement/activity. Body temperatures were monitored with subdermal temperature chips from study Day 57 to the end of study. On the basis of severity of clinical observations (weakness/lethargy, dehydration, nutrition, nasal discharge, ocular discharge, and edema), each condition was classified as mild, moderate or severe, and clinical assessment scores of 1, 2, and 3, respectively, was assigned. An animal adjudged moribund after clinical evaluation was euthanized prior to the scheduled day.

For the assessment of viremia and virus shedding, viral load was evaluated using a qualified qPCR assay targeting the (b) (4)

of MPXV. Assay qualification was performed using a (b) (4) MPXV (b) (4).

Assay qualification report was previously submitted to MF (b) (4) (Appendix K, Amendment 19 to MF (b) (4)) and met qualification specifications. At euthanasia or when a macaque was found dead, gross necropsy was performed and various tissues and organs were collected for examination.

Immunogenicity Results

Results of testing of post-vaccination serum samples for binding antibodies by ELISA and T-cell response by ELISPOT are summarized in Table 5 (adapted from Appendices 1 and 2, and Appendix D of Study Report BN-PRE-12-003 in STN 125678/0).

Table 5. Immunogenicity of MVA-BN in Study BN-PRE-12-003

Group	Vaccination, TCID ₅₀ (Schedule Day)	ELISA peak GMT (% seroconversion)	PRNT peak GMT (% seroconversion)	Mean spot- forming cells/ 3×10^5 PBMCs
1	TBS (0, 28)	1 (0%)	1 (0%)	0
2	MVA-BN LF, 1×10^8 (0, 28)	11777 (100%)	281 (100%)	53
3	MVA-BN (b) (4), 1×10^8 (0, 28)	10099 (100%)	372 (100%)	66

The IgG ELISA results show that vaccinia-specific antibodies were not detected in serum samples obtained from macaques prior to vaccination, as well as in serum samples obtained at

any timepoint from macaques in the TBS treatment group. Two macaques in group 2 seroconverted (IgG titer ≥ 100) on study Day 7 (i.e., one week after the first vaccination) and all macaques vaccinated with MVA-BN (LF (b) (4)) seroconverted on Day 14. On study Day 28, IgG titers ranged from 200 to 2582 in the MVA-BN cohort. Peak IgG GMTs of 11777 (range, 3461 to 34729) and 10099 (range, 4428 to 24805) for MVA-BN LF and MVA-BN (b) (4), respectively, were attained a week after the second vaccination (i.e., Day 35) (Appendix 1, pages 774 to 782 of Study Report BN-PRE-12-003 in STN 125678/0). Results of the PRNT indicate that neutralizing antibody was not detected in any group 1 macaque at any post-vaccination timepoint through Day 56 (Figure 3; page 770 of Study Report BN-PRE-12-003 in STN BL 125678/0).

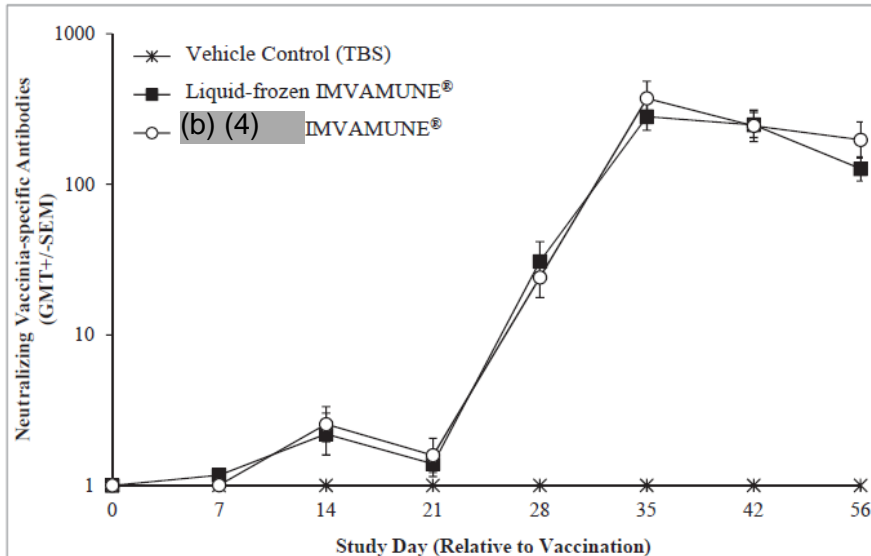


Figure 3. GMT of Neutralizing Antibodies in Macaques in Study BN-PRE-12-003

Two macaques in group 2 and one in group 3 had low titers of neutralizing antibodies on Day 7. The Day 7 PRNT result is confounding for two reasons:

(i). Macaque # A11512 seroconverted on Day 7 with an IgG titer of 200 but had no detectable neutralizing antibodies at this time point. By contrast, macaque #A10746, which had no detectable IgG on Day 7 but had a PRNT titer of 3 at this timepoint (Appendix 2; pages 783 to 791 of Study Report BN-PRE-12-003 in STN 125678/0).

(ii). None of the macaques in group 3 had detectable IgG on Day 7, but macaque # A12582 in the group had a PRNT titer of 3 at this timepoint. Thus, it seems counterintuitive that serum samples with no detectable IgG would contain neutralizing antibody.

On Days 14, 21, and 28, the GMT of neutralizing antibodies in group 2 were 2, 1, and 31, respectively, and the corresponding seroconversion rates were 43%, 21% and 93%. In group 3, PRNT GMTs were 3, 2, and 24 on Days 14, 21, and 28, respectively, with corresponding seroconversion rates of 57%, 21% and 100%. All macaques in groups 2 and 3 had seroconverted by Day 35 (i.e., one week after the second vaccination). PRNT GMT titers also peaked on Day 35, with group 2 PRNT GMT titer of 281 ± 224 SD (range 69 to 372) and group 3 PRNT GMT titer of 372 ± 476 SD (range 48 to 1740) (Appendix 2; pages 783 to 791 of Study Report BN-PRE-12-003 in STN 125678/0). Thus, there was a wide variation in the quantity of neutralizing antibodies detected in the MVA-BN treatment cohort. The PRNT GMT titers on Days 42 and 56 were 248 and 127, respectively, in group 2 and 244 and 197, respectively, in

group 3, indicating a rapid decline in neutralizing antibody after day 42 (i.e., two weeks after the second vaccination).

Results of the IFN- γ ELISPOT assay indicate an absence of IFN- γ producing cells in pre-immunization PBMCs obtained from all macaques. All Day 56 PBMCs that were mock-stimulated with medium as well as group 1 Day 56 PBMCs stimulated with VV-WR had no detectable IFN- γ spots above background (≤ 3 SFC/ 3×10^5 cells) (Appendix D; pages 819 to 820 of Study Report BN-PRE-12-003 in STN 125678/0). All Day 56 PBMC samples from groups 2 and 3 had detectable IFN- γ secreting spots above background. The mean spot forming count (SFC) per 3×10^5 cells in group 2 was 53 (range, 11 to 147 SFC/ 3×10^5 cells) and 66 (range, 12 to 269 SFC/ 3×10^5 cells) for groups 2 and 3, respectively (Table 5).

In summary, immunogenicity data from study BN-PRE-12-003 indicate that the MVA-BN induces both humoral (including neutralizing antibodies) and cellular immune responses against vaccinia virus. The seemingly wide variation in the individual immune responses, particularly T-cell responses, is typical. A similar pattern was observed in control-cell populations stimulated with concanavalin A, a mitogen that non-specifically stimulates T cells, where the number of IFN- γ secreting cells ranged from 201 to > 1500 SFC/ 3×10^5 cells.

Results of MPXV Challenge (MPXV Morbidity and Survival)

Following MPXV challenge on Day 63, mild clinical symptoms were observed in the TBS group between Day 65 to 69 (two to six days post-challenge). Clinical symptoms, including hunched posture, inappetence became moderate to severe and dehydration was mild-to-moderate. A macaque in the TBS group was found dead on Day 72 (i.e., 9 days after challenge). Two other macaques in the group met criteria for euthanasia on Day 78, and three others were adjudged to be euthanized on Day 78. Thus, a total of six macaques in the placebo group succumbed to MPXV infection and four macaques survived (pages 201 to 203, and Table 6 (adapted from Appendices F, G and J) of Study Report BN-PRE-12-003 in STN 125678/0).

Of the four survivors, two experienced mild disease (low clinical scores, low lesion counts, transient body weight loss, low) in spite of being seronegative (ELISA and PRNT) prior to exposure to MPXV. The applicant claimed that this observation may be due to residual immunological memory that was not detectable in the pre-screening antibody assays or may be due to animal-to-animal variations. The former explanation appears more plausible since a dose of 5×10^7 PFU of MPXV inoculated intravenously in naïve cynomolgus macaques is mostly lethal. Three of the group 1 macaques that succumbed had severe dehydration, and all six that succumbed and two survivors in the group had moderate-to-severe edema.

Table 6. MPXV Morbidity and Mortality in Study BN-PRE-12-003

Group	Vaccination	No. of macaques	Peak Mean Total Body Lesion Count (Range)	Peak Mean Blood MPXV Load (genome copies/mL)	Mean Peak Throat MPXV Shedding (genome copies/mL)	Survival (%)
1	TBS	10	707.8 (77 - >1000)	9.8×10^7	1.5×10^8	4/10 (40)
2	MVA-BN LF	14	104.7 (3 - 305)	5.8×10^4	1.6×10^6	14/14 (100)
3	MVA-BN ^{(b) (4)}	14	63.6 (1 - 195)	5.8×10^3	3.9×10^5	14/14 (100)

Nasal/ocular discharge was recorded in four group 1 macaques. Mean clinical assessment scores were highest in group 1 macaques, peaking at 8.7 on Day 76 and dropping to 3.5 in the four surviving macaques on Day 80 (Figure 4, page 593 and Appendix F (pages 585 to 593) of Study Report for BN-PRE-12-003 in STN BL 125678/0).

All macaques vaccinated with MVA-BN (groups 2 and 3) survived MPXV challenge, with lower incidence of severe disease when compared to the TBS treatment group. Clinical signs (hunched posture, inappetence, dehydration) were milder in groups 2 and 3, with moderate dehydration occurring in about half the number of macaques in each group; moderate edema in four group 2 macaques, with one becoming severe. All clinical signs had resolved 18 to 21 days after challenge. Mean clinical assessment scores were generally lower in the MVA-BN treatment groups, peaking at 2.9 on Days 73 and 75 and dropping to 0.6 on Day 80. Similarly,

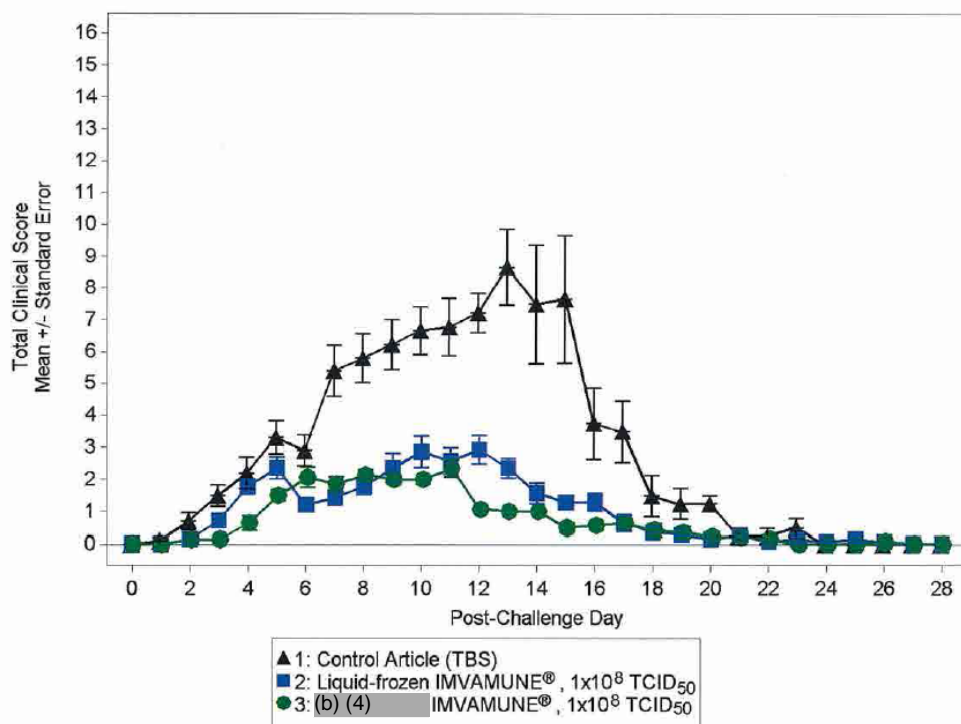


Figure 4. Total Clinical Scores After MPXV Challenge of Macaques in Study BN-PRE-12-003

mean clinical scores in group 3 peaked at 2.4 on Day 74 and dropped to 0.6 on Day 80 (Figure 4). There was no apparent correlation between morbidity and weight and/or gender.

Body Weight and Body Temperature

Macaques in the TBS group lost weight between Days 66 to 81 with a mean peak weight loss of 8.8% (range, 5.9% to 19.6%) on Day 78. Except for macaque #A09862, all group 2 macaques experienced a transient weight loss of between 1.9 to 5.7% from Day 66 to 72. In group 3, only three (3/14) macaques experienced body weight loss of between 2.2% to 6.7%. The applicant's analysis indicates a statistically significant difference in weight loss between groups 1 and 2 ($p \leq 0.045$, BN's analysis) and between group 1 and group 3 from Day 66 and 81 ($p \leq 0.0068$; BN's analysis) from Day 72 to 81.

Mean body (shoulder) temperature changes (from baseline) in TBS group were higher than the MVA-BN groups from Day 65 to 68, but generally fluctuated, particularly in readings obtained from hip chips.

Pock Lesions

Monkeypox lesions developed in all macaques in the TBS group from Day 69, with a mean total body count of 401.9 lesions. At this time point, 4/10 macaques had pock lesions TNTC (i.e., lesion > 200) on at least one region (arm, leg or ventral torso). Seven of ten (7/10) macaques in group 1 had lesions TNTC in at least one area of the body from Day 69 to Day 81. The mean terminal total body lesion count for the six group 1 macaques that succumbed was 922. Macaque #s A10235 and A09980 in group 1 had peak counts of 77 and 155 on days 75 and 78, respectively. A peak mean total body lesion count of 707.8 (range, 77 to >1000) was recorded for group 1 on Day 75 (Table 6; Appendix G (pages 597 to 601); and Figure 5 (page 602) of Study Report BN-PRE-12-003 in STN BL 125678/0). Interestingly, macaque # A09812 had hemorrhagic TNTC lesions on the legs and countable hemorrhagic lesions on the head from Day 69 to 81, and macaque #A10916 had hemorrhagic TNTC lesions on the arms and legs from Day 72 to 75, but both survived. By contrast, macaque # A12225 had a mean peak total body lesion counts of 532 (Day 72) and did not survive. Macaque A12225 succumbed on Day 72. The four surviving macaques in group 1 had a daily mean total body lesion count < 75 from Day 84 but no lesions were present by Day 91.

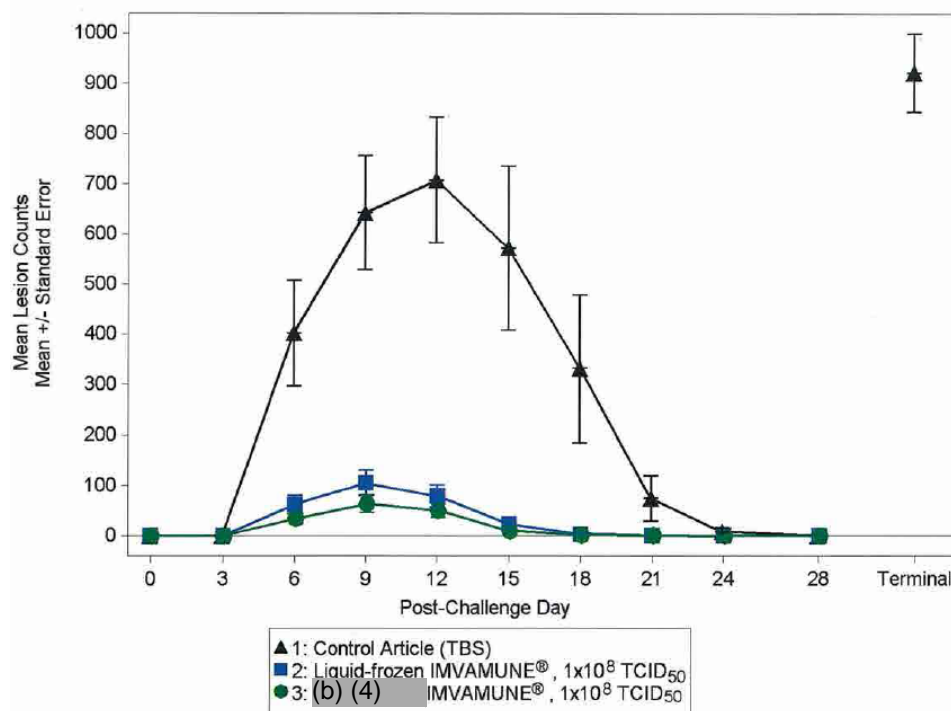


Figure 5. Group Mean Lesion Counts After MPXV Challenge of Macaques in Study BN-PRE-12-003. The Mean Terminal Viral Load for Macaques That Succumbed in Group 1 is Shown as “Terminal”.

Among MVA-BN vaccinated macaques, two macaques in group 2 and 11 macaques in group 3 had monkeypox lesion on Day 69, with mean total body counts of 62.1 and 34, respectively.

None of the macaques in the MVA-BN cohort had lesions TNTC at any timepoint. Peak mean total body lesion count was 104.7 (range, 3 to 305) and 63.6 (range, 1 to 195) in groups 2 and 3, respectively; both recorded on Day 72. From Day 78, the mean total body lesion count was < 25 in group 2 and < 10 in group 3, and with the exception of a macaque that had a total count of 30 lesions on Day 81, all other macaques in the MVA-BN cohort had total lesion counts of between 0 to 4, and no lesions were detected after Day 84. According to the applicant's analysis, pock lesion counts were significantly higher in the TBS group than the MVA-BN cohort from Day 69 to 81 ($p \leq 0.00053$ by BN's analysis). Although pock lesion development in the TBS group did not seem to accurately predict fatality outcomes, overall pock lesion counts were higher in the TBS group, implying efficacy of MVA-BN in protecting against MPXV morbidity in this model.

Blood Viral Load (Viremia)

Mean blood viral load peaked on Days 72 and 75 in the TBS group, with 9.8×10^7 (range 3.3×10^6 to 1.5×10^9) genome copies/mL detected at both timepoints (Table 6, and Figure 6 (page 640) of Study Report BN-PRE-12-003 in STN 125678/0). Two of the four group 1 survivors cleared MPXV by Day 91 and the remaining two survivors had 1.1×10^3 to 3.2×10^3 genome copies/mL. In group 2, a mean peak MPXV load of 5.8×10^4 genomes/mL was recorded on Day 72 (range, 1.9×10^3 to 1×10^6 genome copies/mL). In group 3, peak viremia

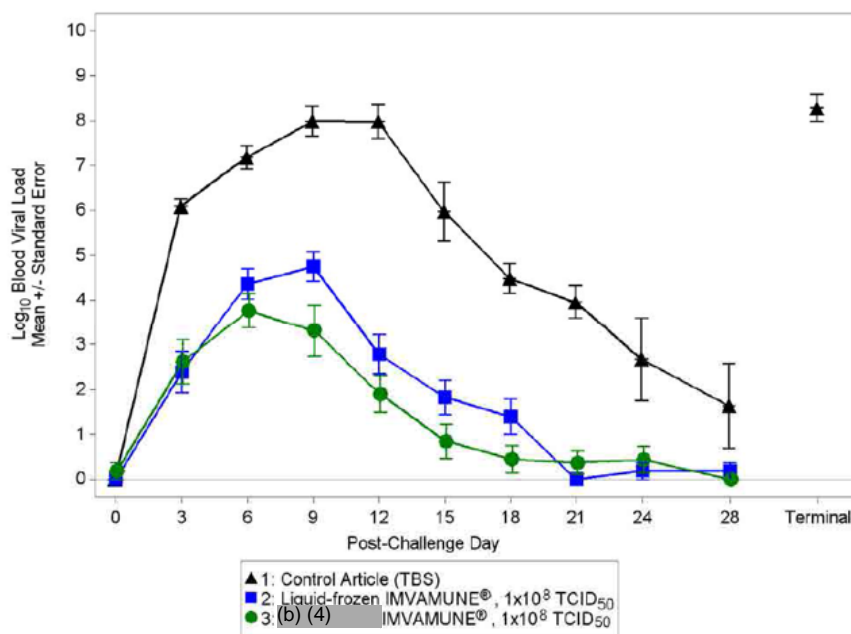


Figure 6. Group Mean (Log₁₀) Blood Viral Load After MPXV Challenge of Macaques in Study BN-PRE-12-003. The Mean Terminal Viral Load for Macaques That Succumbed in Groups 1 is Shown as “Terminal”.

was recorded on Day 69, with 13/14 macaques having viremia at a mean of 5.8×10^3 genome copies/mL (range, 4×10^2 to 1.5×10^5 genome copies/mL). Except for one macaque in group 2, all macaques vaccinated with MVA-BN had no detectable MPXV genome by the end of the study.

The applicant's analysis indicated a statistically significant difference ($p \leq 0.000036$) in blood viral load between the TBS group and MVA-BN cohort at every timepoint from Day 66 through Day 91. MPXV genome was detected in all four surviving unvaccinated macaques (group 1) through Day 84 (Day 84 range, 1.6×10^3 to 8.7×10^4 genome copies/mL), the majority of macaques (26/28) in the MVA-BN cohort had no detectable MPXV genome at this timepoint; the remaining two macaques (both in group 3) had MPXV genome copy numbers below the limit of quantification of 7.9×10^2 genome copies/mL. This set of data indicate effective clearance of circulating MPXV in macaques vaccinated with MVA-BN.

Throat Virus Shedding

MPXV genome was detected in throat swab samples of all macaques in the TBS group and in 23/28 of MVA-BN vaccinated macaques on Day 66. With the exception of group 3 macaque # A12582, macaques in all treatment groups were shedding MPXV by Day 69. Macaque # A12582 had no detectable MPXV genome at most timepoints except for a transient 6.3×10^3 genome copies/mL recorded on Day 75. Mean peak virus shedding was recorded in all treatment groups on Day 72. The mean peak MPXV shedding for the TBS group was 1.5×10^8 (range, 2.1×10^7 to 1.5×10^9) genome copies /mL. For groups 2 and 3, mean peak virus shedding were 1.6×10^6 (range, 5.4×10^4 to 8.5×10^6) genome copies /mL, and 3.9×10^5 (range, 8.3×10^1 to 1.5×10^8) genome copies /mL, respectively (Table 6, and Figure 7 (page 644) of Study Report BN-PRE-12-003 in STN BL 125678/0).

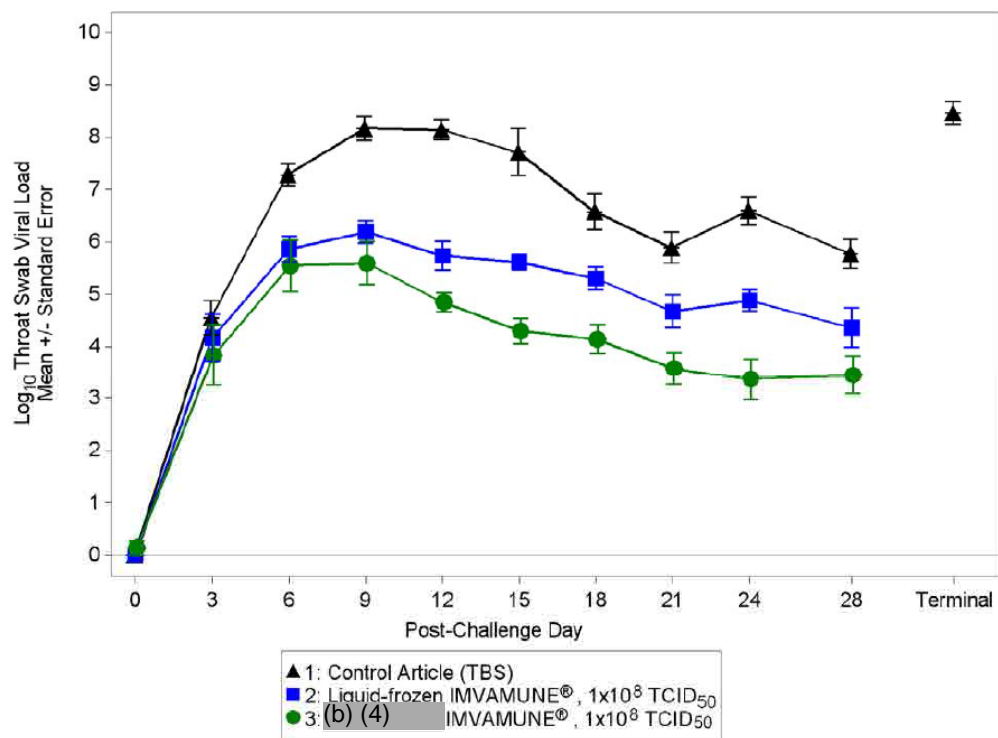


Figure 7. Group Mean (Log₁₀) Throat Swab Viral Load After MPXV Challenge of Macaques in Study BN-PRE-12-003. The Mean Terminal Viral load for Macaques That Succumbed in Group 1 is Shown as “Terminal”.

Mean virus shedding declined in all groups after Day 72, but virus shedding continued in all treatment groups through the end of study except in group 3 macaque #A12582. The applicant's analysis shows a statistically significant difference ($p \leq 0.0078$) in virus shedding between the TBS group and the MVA-BN vaccinated cohort from Day 69 to the termination of the study. From Day 78 to 87, significantly lower virus shedding ($p \leq 0.03$) was observed in macaques in group 3 than those in group 2..

Thus, virus shedding evaluation indicated that MPXV was not completely cleared in all treatment groups, irrespective of vaccination treatment. However, virus shedding was comparatively at lower levels in MVA-BN vaccinated macaques than in the TBS group, suggesting that MVA-BN vaccination was effective in reducing virus shedding.

Pathology

In group 1 macaques that succumbed to MPXV infection gross lesions consistent with monkey pox, including pock lesions on epithelial surfaces (skin, tongue and large intestine) were observed. Mottled discoloration of the lungs, and/or enlargement of the thymus, tonsils and/or multiple lymph nodes were also recorded. In surviving macaques, gross lesions were limited to crusts, pocks, and/or scars on the skin, lungs, and tongue, as well as lymph node enlargement that suggested reactive lymphoid hyperplasia.

Review comment: The results from study BN-PRE-12-003 indicated that both LF (b) (4) formulations of MVA-BN induced high titers of binding and neutralizing antibodies, as well as T-cell responses in macaques. Macaques vaccinated with MVA-BN were protected from monkeypox morbidity after intravenous MPXV challenge as evidenced in the relatively lower numbers of pock lesions, clinical scores, and lower levels of MPXV viremia and throat virus shedding, when compared to placebo-treated macaques. Further, a combined total of 28 macaques vaccinated with MVA-BN in this study survived MPXV challenge (i.e., 100% survival). By contrast, a 60% mortality was recorded in unvaccinated macaques and the four survivors in the placebo group developed more severe disease than vaccinated macaques. Thus, consistent with Study BN-PRE-08-004, data from this study suggest an enhanced survival benefit in macaques vaccinated with MVA-BN in the NHP/MPXV intravenous challenge model.

Study BN-PRE-08-005 (NHP/MPXV Intratracheal Challenge)

Title

Comparison of the Immunogenicity and Protective Efficacy of IMVAMUNE® and ACAM2000™ in the Intratracheal Monkeypox Challenge Model in Cynomolgus Monkeys

Study Design Summary

Study BN-PRE-08-005 was conducted as Study No. 12425.01b at SRI, Frederick, Maryland. The study was identical to Study BN-PRE-08-004 (above) with regard to treatment groups and vaccination schedule, except that on Day 63, macaques were challenged with 5×10^6 PFU of

MPXV via the intratracheal route (Table 7; adapted from Table 2 of Study Report BN-PRE-08-005 in STN 125678/0). The study aimed to evaluate the immunogenicity and protective efficacy of the candidate MVA-BN smallpox vaccine in comparison with licensed ACAM2000 smallpox vaccine in a MPXV intratracheal challenge model in cynomolgus macaques. Vaccinated macaques and an unvaccinated control group were challenged with MPXV (intratracheal route) and evaluated for monkeypox morbidity and mortality post-MPXV exposure.

Clinical-grade MVA-BN LF formulation (lot # 0030707) and TBS (lot # (b) (4)) were supplied by the applicant. ACAM2000 (lot # VV04-003-A) and ACAM2000 diluent (lot # (b) (4)) were obtained from the CDC (Atlanta, Georgia). Lot (b) (4) of MPXV (b) (4), was obtained from (b) (4).

Table 7. Design of Study BN-PRE-08-005

Group	No. of macaques	Vaccination	Dose	Route	Vaccination schedule (study day)	MPXV challenge on day 63 (PFU)
1	3	TBS	N/A	SC	0, 28	5×10^6
2	5	MVA-BN	1×10^6 TCID ₅₀	SC	0, 28	5×10^6
3	5	MVA-BN	1×10^7 TCID ₅₀	SC	0, 28	5×10^6
4	5	MVA-BN	1×10^8 TCID ₅₀	SC	0, 28	5×10^6
5	6	MVA-BN	1×10^8 TCID ₅₀	SC	28	5×10^6
6	6	ACAM2000	2.5- 12.5×10^5 PFU	PC	28	5×10^6

N/A, Not Applicable; SC, subcutaneous; PC, percutaneous

Thirty (30) specific pathogen-free cynomolgus macaques aged 2-5 years and weighing 2.81-4.09 kg were sourced from (b) (4). After quarantine, animals were randomized into six groups (Table 7). On study Days 0 and 28, three groups-of-five macaques were vaccinated with 1×10^6 , 1×10^7 or 1×10^8 TCID₅₀ of MVA-BN, respectively. A group-of-six macaques received 1×10^8 TCID₅₀ of MVA-BN on Day 28, and another group-of-six was vaccinated with 2.5- 12.5×10^5 PFU of ACAM2000 on Day 28. A control group-of-three macaques received TBS on Days 0 and 28.

Serum samples collected 14 days before vaccination, as well as on study Days 0, 7, 14, 21, 28, 35, 42, and 56, were tested for total IgG and neutralizing antibodies. Additional blood samples for antibody array were obtained on Day 56.

On Day 63, each macaque was anesthetized by intramuscular injection of ketamine (10-30 mg/kg), xylazine (0.35 mg/kg) and atropine (0.04 mg/kg) and challenged by delivery of 5×10^6 PFU of MPXV (in 1 mL suspension) into the mid-to-distal trachea using a flexible bronchofiberscope. Additional blood samples for immunoassays were collected on post-challenge Days 69, 75, 81, 87 and 91. Blood samples for the assessment of viremia by real-

time qPCR targeting the MPXV ^{(b) (4)} gene were collected on Days 63, 69, 72, 75, 78, 81, 84, 87, and 91. Following MPXV challenge, animals were evaluated for morbidity and mortality as described under Study BN-PRE-08-004.

Immunogenicity Results

All macaques vaccinated with ACAM2000 developed a pock lesion (vaccine take) that peaked on Day 35 (i.e., 7 days post-vaccination). Pock lesion sizes ranged from 0.058 to 1.005 cm² (mean = 0.39 ± 0.36 SD), with two macaques developing small lesion sizes. The antibody data show, however, that while macaque # 4530 with the largest lesion size of 1.005 cm² had a peak IgG titer of 3323 and PRNT titer of 196 on Day 56, macaque # 4540 with a lesion size of 0.39 cm² had an IgG titer of 23754 and PRNT titer of 1016 at the same timepoint. This suggests a possible lack of correlation between lesion size and the induced antibody response. Macaques in the MVA-BN cohort or TBS group did not develop a vaccine take at any timepoint.

The immunogenicity data are summarized in Table 8 (adapted from Table 4, Table 5 and Appendices 1 and 2 of Amendment 1 to Study Report BN-PRE-08-005 in STN 125678/0). Except for serum samples from macaque # 4535 in group 2 and # 4532 in group 5, all pre-vaccination serum samples had no detectable binding IgG (Appendix 1 (pages 28 to 36) of Amendment 1 to STN 125678/0). The two exceptions were considered to be falsely positive (macaque # 4532 had an IgG titer of 100 on both Day 7 and 28). Serum samples from the TBS group had no vaccinia-specific IgG at any timepoint. In group 2, a macaque was seropositive (IgG titer = 100) on Day 7. Similarly, 3/5, and 4/5 macaques in groups 3 and 4, respectively, were seropositive on Day 7 (IgG titer range 100 to 533). All macaques in groups 3 and 4 had seroconverted by Day 14 (Table 8; page 18 of Amendment 1 to Study BN-PRE-08-005 in STN 125678/0), with IgG GMTs of 525 (titer range, 200 to 890) and 756 (titer range, 400 to 1388), respectively.

Table 8. Immunogenicity of MVA-BN Smallpox Vaccine in Study BN-PRE-08-005

Group	Vaccination (Schedule Day)	Peak ELISA GMT (% seroconversion)	Peak PRNT GMT (% seroconversion)
1	TBS (0, 28)	1 (0)	1 (0)
2	1x10 ⁶ TCID ₅₀ (0, 28)	866 (100)	179 (100)
3	1x10 ⁷ TCID ₅₀ (0, 28)	5748 (100)	891 (100)
4	1x10 ⁸ TCID ₅₀ (0, 28)	18816 (100)	1287 (100)
5	1x10 ⁸ TCID ₅₀ (28)	1467 (100)	120 (100)
6	2.5-12.5x10 ⁵ PFU ACAM2000 (28)	2020 (100)	198 (100)

Peak IgG titers were attained in groups 2 to 4, seven days after the booster dose (i.e., Day 35), and all macaques in the MVA-BN prime/boost treatment groups had seroconverted. Peak IgG GMTs for groups 2, 3, and 4 were 866 (range, 571 to 1477), 5748 (range, 4363 to 14389), and 18816 (range, 6729 to 37984), respectively (Table 8, and Appendices 1 and 2 of Amendment 1 to Study Report BN-PRE-08-005 in STN 125678/0). In the single-dose MVA-BN group, a peak GMT of 1467 (range, 712 to 2895) was recorded on Day 42 (i.e., 14 days after vaccination). A GMT of 2020 (range, 423 to 23754) was obtained for the ACAM2000 group on Day 56, which cannot be interpreted as the peak GMT since there were no other IgG measurement timepoints beyond Day 56. Among the MVA-BN prime/boost treatment cohort, the IgG data

indicate a linear relationship between dose and the IgG response (applicant's analysis: $R^2 = 0.875$; Spearman rank test p -value = 0.0000026) (page 19 of Amendment 1 to STN 125678/0).

Table 9. Geometric Mean Titers of ELISA Antibodies in Study BN-PRE-08-005

	Group 1			Group 2			Group 3			Group 4			Group 5			Group 6		
	TBS			IMVAMUNE® 1x10 ⁶			IMVAMUNE® 1x10 ⁷			IMVAMUNE® 1x10 ⁸			IMVAMUNE® 1x10 ⁸ (once)			ACAM2000™		
Day	% ¹	GMT ²	N ³	% ¹	GMT ²	N ³	% ¹	GMT ²	N ³	% ¹	GMT ²	N ³	% ¹	GMT ²	N ³	% ¹	GMT ²	N ³
-14	0	1	3	0	1	5	0	1	5	0	1	5	0	1	6	0	1	6
0	0	1	3	0	1	4	0	1	5	0	1	5	0	1	6	0	1	6
7	0	1	3	20	3	5	60	22	5	80	74	5	0	1	5	0	1	6
14	0	1	3	20	3	5	100	525	5	100	756	5	0	1	6	0	1	6
21	0	1	3	40	7	5	100	460	5	100	1010	5	0	1	6	0	1	6
28	0	1	3	80	53	5	100	565	5	100	1028	5	0	1	5	0	1	6
35	0	1	3	100	866	5	100	5748	5	100	18816	5	83	164	6	0	1	6
42	0	1	3	100	655	5	100	3388	5	100	13830	5	100	1467	6	100	728	6
56	0	1	3	100	291	5	100	1997	5	100	7778	5	100	1190	6	100	2020	6

Animals in Group 1 to 4 were vaccinated (s.c.) with either TBS or the various doses of IMVAMUNE® on Day 0 and 28. Animals in Group 5 and 6 were vaccinated with either IMVAMUNE® (s.c.) or ACAM2000™ (p.c.) on Day 28.

¹Seroconversion. ²Geometric mean titer. ³Number of animals.

Macaques in all groups were seronegative for neutralizing antibodies pre-vaccination. All macaques in groups 3 and 4 had neutralizing antibodies on Day 7 (one week after the first vaccination) with PRNT GMTs of 22 (range, 6 to 346) and 140 (range, 46 to 415), respectively (Appendix 2 (pages 29 to 45) of Amendment 1 to STN 125678/0). At the equivalent timepoint for group 5 (single-dose MVA-BN) 83% PRNT seroconversion with a GMT of 19 (range, 1 to 66) was recorded, and 1/6 (17%) macaques in the ACAM2000 treatment group had neutralizing antibodies (titer = 6) (Table 10; page 21 of Amendment 1 to Study BN-PRE-08-005 in STN 125678/0). Similar to the observation under study BN-PRE-12-003, macaque # 4527 in

Table 10. Geometric Mean Titers of Neutralizing Antibodies in Study BN-PRE-08-005

	Group 1			Group 2			Group 3			Group 4			Group 5			Group 6		
	TBS			IMVAMUNE® 1x10 ⁶			IMVAMUNE® 1x10 ⁷			IMVAMUNE® 1x10 ⁸			IMVAMUNE® 1x10 ⁸ (once)			ACAM2000™		
Day	% ¹	GMT ²	N ³	% ¹	GMT ²	N ³	% ¹	GMT ²	N ³	% ¹	GMT ²	N ³	% ¹	GMT ²	N ³	% ¹	GMT ²	N ³
-14	0	1	3	0	1	5	0	1	4	0	1	5	0	1	5	0	1	6
0	0	1	3	0	1	5	0	1	5	0	1	5	0	1	6	0	1	6
7	0	1	3	0	1	5	100	22	5	100	140	5	0	1	6	0	1	6
14	0	1	2	60	5	5	100	202	5	100	355	5	0	1	6	0	1	6
21	0	1	3	40	3	5	100	74	5	100	56	5	0	1	6	0	1	6
28	0	1	3	0	1	5	100	11	5	100	19	5	0	1	6	0	1	6
35	0	1	3	100	179	5	100	891	5	100	1287	5	83	19	6	17	1	6
42	0	1	3	100	18	5	100	115	5	100	416	5	67	9	6	100	66	6
56	0	1	3	100	53	5	100	216	5	100	470	5	100	120	6	100	198	6

Animals in Group 1 to 4 were vaccinated (s.c.) with either TBS or the various doses of IMVAMUNE® on Day 0 and 28. Animals in Group 5 and 6 were vaccinated with either IMVAMUNE® (s.c.) or ACAM2000™ (p.c.) on Day 28.

¹Seroconversion. ²Geometric mean titer. ³Number of animals

group 3 with an IgG titer of 100 on Day 7 had a PRNT titer of 346 at the same timepoint. Macaque # 4523 with the same IgG titer (100) in the same group had a PRNT titer of 75, and macaque # 4542 with the highest Day 7 IgG titer of 533 had a PRNT titer of 6 (same titer as in two other macaques with no detectable IgG). Peak neutralizing antibody titers of 179 (range, 75 to 262), 891 (range, 751 to 1361), and 1287 (range, 928 to 1716), were obtained on Day 35 (seven days after boost) for macaques in groups 2, 3, and 4, respectively (Table 8). In group 5, 100% PRNT seroconversion was obtained on Day 56 (28 days post-vaccination), differing from the post-priming response in group 4 (100% seroconversion on Day 7). The peak PRNT GMTs for groups 5 and 6 were 120 (range, 43 to 224) and 198 (range, 103 to 1016), respectively, both obtained on Day 56. It is not known if the Day 56 GMTs represent the peak PRNT GMTs for the single-dose MVA-BN and ACAM2000 treatment groups since there were no PRNT data beyond the Day 56 timepoint. The correlation between dose and PRNT GMTs was not as strong (applicant's analysis: $R^2 = 0.6575$; Spearman rank test p -value = 0.00033) (page 21 of Amendment 1 to STN 125678/0). Although the applicant concluded that there is a strong correlation between ELISA titers and PRNT titers (no analysis was performed) but data from this study did not support such a correlation. Notwithstanding, the immunogenicity data from this study indicate that MVA-BN induced vaccinia-specific antibody responses in cynomolgus macaques, including neutralizing antibodies and may be useful in protecting against MPXV infection in humans.

Results of MPXV Challenge (MPXV Morbidity and Survival)

All TBS group macaques developed respiratory distress (dyspnea, coughing (2/3), or nasal discharge (1/3)), moderate-to-severe anorexia, dehydration, and depression (2/3). A macaque was euthanized on each of Day 6 and 7. Group 1 macaque # 4525 was severely diseased (highest pock lesion count in the study (56 lesions on Day 78) and the only viremic macaque at all timepoints) but survived through Day 91. Two macaques in group 2 developed moderate to

Table 11. MPXV Morbidity and Mortality in Study BN-PRE-08-005

Group	Vaccination (Schedule Day)	No. of macaques	Peak Mean Total Body Lesion Count	Peak Mean Blood MPXV Load (genome copies/mL)	No of macaques without viremia at any timepoint	Survival (%)
1	TBS (0, 28)	3	3 ^a	1.2x10 ⁶	0/3	1/3 (33)
2	1x10 ⁶ TCID ₅₀ (0, 28)	5	4.4	2.2x10 ⁶	0/5	3/5 (60)
3	1x10 ⁷ TCID ₅₀ (0, 28)	5	3.8	1.4x10 ⁵	1/5	5/5 (100)
4	1x10 ⁸ TCID ₅₀ (0, 28)	5	4.8	3.2 x10 ⁴	1/5	5/5 (100)
5	1x10 ⁸ TCID ₅₀ (28)	6	4.7	8.7x10 ⁵	0/6	5/6 (83)
6	2.5-12.5x10 ⁵ PFU ACAM2000 (28)	6	4.3	^b < 5x10 ³	6/6	6/6 (100)

^aData for day 69, the surviving macaque had a peak lesion count of 56 on Day 78

^bAll macaques were below the limit of detection of 5x10³ genome copies/mL.

severe respiratory distress and had to be euthanized on Day 9. All vaccinated survivors, irrespective of group, experienced dehydration after MPXV challenge but no other clinical signs of monkeypox. All macaques in groups 3, 4, and 6 survived (Table 11; adapted from Table 4 (page 24) and Appendices 6 & 7, of Study Report BN-PRE-08-005 in STN 125678/0). A macaque in group 5 (# 4529; single-dose MVA-BN) developed severe respiratory distress and was euthanized on Day 9. Macaque # 4529 had the second highest IgG titer (1339) in group 5, seven days post-vaccination and a PRNT titer of 53 on Day 56. Based on the IgG and PRNT data (Table 8), MVA-BN induced higher antibody titers and at an earlier timepoint than ACAM2000. However, consistent with data from study BN-PRE-08-004, ACAM2000 was more effective in preventing MPXV morbidity in macaques suggesting possible differences in the quality of immune responses.

Body Weight and Body Temperature

All macaques, irrespective of treatment group, experienced a transient loss in body weight from Day 69 to 72 (Figure 8; page 26 of Study Report BN-PRE-08-005 in STN 125678/0). Weight loss was more severe in the TBS group, with peak losses of 9.5% and 13.2% for macaque #s 4521 and 4534, respectively, at the point of euthanasia (Appendix 4; pages 53 to 57 in Study Report BN-PRE-08-005 of STN 125678/0). Macaque #4525 lost 9.0% at peak on Day 72 but was close to baseline by Day 87 (Day 87 weight was 99.7% of original weight on Day 63). The three survivors in group 2 were above or at baseline between Day 78 to 87. Similarly, group 3 macaques were above or at baseline weight levels between Day 75 to 91. In group 4, 80% was above or at baseline from Day 72 to 78, but macaque # 4524 (with peak IgG and PRNT titers of 15726 and 1431 on Day 35, respectively) remained at about 5% weight loss through Day 91. The five survivors in group 5 were at or above baseline from Day 72 to 84. Half (3/6) of macaques in the ACAM2000 group were above or at baseline by Day 69 to 72 and two others on Day 78 and 81. Group 6 macaque # 4533 (Day 56 PRNT titer = 129) remained at about 5% below baseline through Day 91.

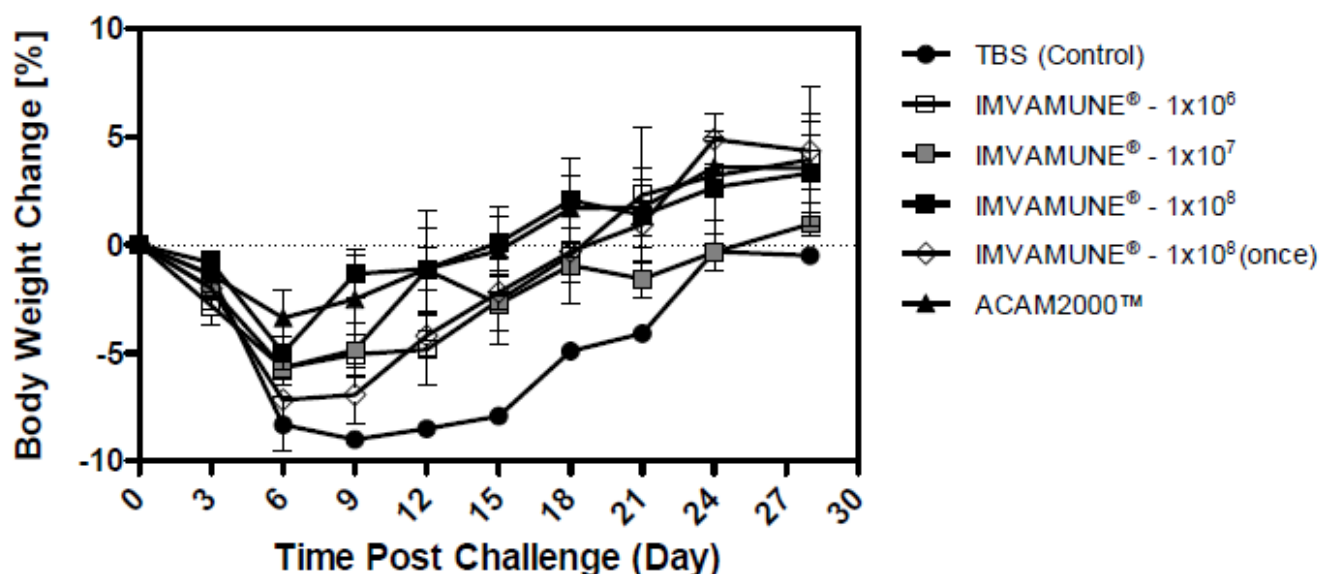


Figure 8. Mean Body Weight Changes After MPXV Challenge in Study BN-PRE-08-005

Body temperature changes were not apparent in the two group 1 macaques that succumbed to MPXV infection. The surviving group 1 macaque had a 3.2% increase in temperature on Day 72 and returned to baseline on Day 75 (Appendix 5; pages 60 to 64 in Study Report BN-PRE-08-005 of STN 125678/0). Most macaques in the MVA-BN groups 2 and 5 experienced a $\leq 2.3\%$ increase in body temperature between Day 66 to 72. The three macaques euthanized on Day 9 were between 1.6% to 3% below baseline in body temperature. All macaques in groups 3, 4, and 6 experienced body temperature changes of $\leq 1\%$ below baseline through Day 91.

Pock Lesions

In the TBS group, macaque # 4521 that was euthanized on Day 69 had no visible pock lesions. Macaque 4534 had 4 pocks from Day 66 till it was euthanized on Day 70 (Appendix 7; pages 72 to 74 in Study Report BN-PRE-08-005 of STN 125678/0). The group 1 survivor had 5 pocks on Day 66 that gradually increased peaking at 56 on Day 78, but lesions were cleared by Day 87. In all vaccinated macaques, irrespective of treatment group, pock lesion counts were extremely low, with majority between 0 to 6 lesions throughout the post-challenge period. Macaque # 4538 in group 4 had a peak count of 20 lesions (the highest among vaccinated macaques) on Day 75. Unlike intravenous MPXV challenge, pock lesion development does not appear to be a prominent feature of MPXV pathogenesis in the intratracheal challenge model in cynomolgus macaques.

Blood Viral Load

In the two macaques euthanized in group 1, peak viremia was recorded on Day 69 with viral loads of 1×10^7 and 1×10^6 genome copies/mL for macaque #4521 and #4534, respectively. The lone survivor in the group had a peak load of 3.9×10^5 genome copies/mL on Day 72 but was viremic through the end of the study. A group mean of 1.2×10^6 genome copies/mL (Table 10) was recorded at peak on Day 69. In group 2, a mean of 2.2×10^6 genome copies/mL was recorded on Day 72. One of the macaques euthanized at this timepoint had a load of 1.1×10^7

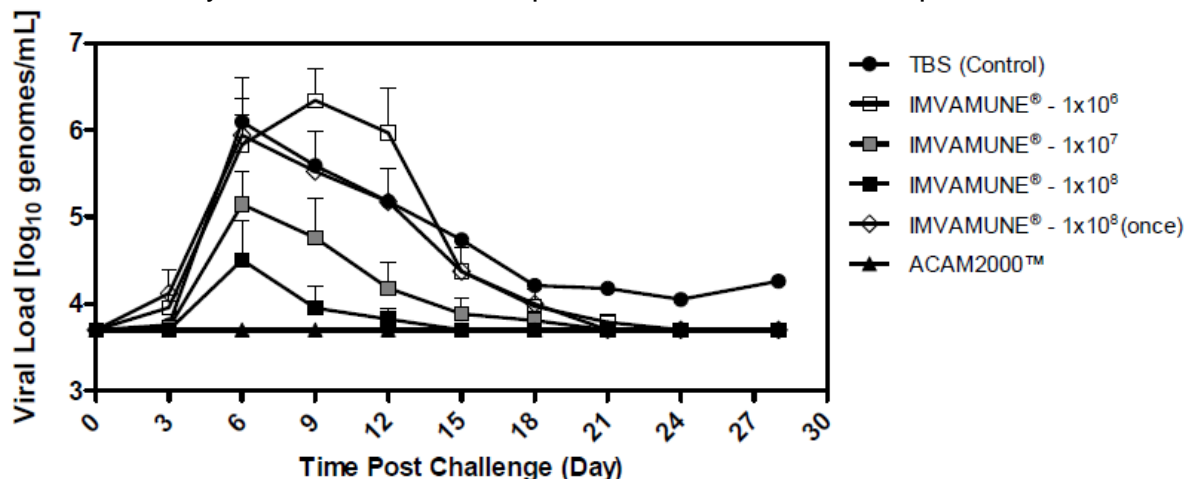


Figure 9. Mean Blood Viral Load Following MPXV Challenge in Study BN-PRE-08-005

genome copies/mL and the second macaque had peaked at 1.5×10^6 on Day 6 prior to succumbing on Day 72. Macaque # 4535 had a peak load of 1×10^7 on Day 72 but survived

with MPXV below detection level by Day 87. Among group 3 macaques, peak viremia was recorded on Day 69 (mean at peak = 1.4×10^5 genome copies/mL). Macaque #4527 in the group was below detection level throughout the post-MPXV challenge period. All group 3 macaques were below detection level by Day 84 (Figure 9; page 30 of Study Report BN-PRE-08-005 in STN 125678). One macaque had below LOD level of MPXV genome copies in group 4. The remaining four macaques had transient viremia at least at one timepoint. A group 4 mean load of 3.2×10^4 genome copies/mL was recorded on Day 69, but all were below the LOD from Day 78. All single-dose MVA-BN vaccinated macaques (group 5) had viremia with a mean of 8.7×10^5 genome copies/mL on Day 69. Complete MPXV clearance in the five survivors in group 5 was recorded on Day 84. All six macaques vaccinated with ACAM2000 (group 6) had no detectable MPXV genome at any timepoint.

Review comment: All macaques vaccinated with MVA-BN by prime/boost inoculation at an interval of 28 days, like ACAM2000-vaccinated macaques, were protected from monkeypox fatality following intratracheal challenge with MPXV. MVA-BN also conferred reduced MPXV pathogenesis when compared to unvaccinated macaques. This is consistent with the data obtained in study BN-PRE-08-004.

Study BN-PRE-09-003 (NHP/MPXV Intratracheal Challenge)

Title

Comparison of the Immunogenicity and Protective Efficacy of IMVAMUNE® and ACAM2000™ in the Intratracheal Monkeypox Challenge Model in Cynomolgus Monkeys

Study Design Summary

Study BN-PRE-09-003 was conducted at SRI (SRI study # 12735.01). The study was similar in objective to Study BN-PRE-08-005 (above) but was more streamlined to compare MVA-BN at the proposed clinical dose of 10^8 TCID₅₀ (prime/boost), as well as a single dose of MVA-BN, with ACAM2000 and an unvaccinated control arm (Table 12; adapted from Table 2 of Study Report BN-PRE-09-003 in STN 125678/0). Vaccinated macaques and unvaccinated macaques were challenged with MPXV via the intratracheal route and evaluated for monkeypox morbidity and mortality post-challenge.

Table 12. Design of Study BN-PRE-09-003

Group	No. of macaques	Vaccination	Dose	Route	Vaccination schedule (study day)	MPXV challenge on day 63 (PFU)
1	8	TBS	N/A	SC	0, 28	5×10^6
2	10	MVA-BN	1×10^8 TCID ₅₀	SC	0, 28	5×10^6
3	10	MVA-BN	1×10^8 TCID ₅₀	SC	28	5×10^6
4	10	ACAM2000	$2.5\text{--}12.5 \times 10^5$ PFU	PC	28	5×10^6

N/A, Not Applicable; SC, subcutaneous; PC, percutaneous

Clinical-grade MVA-BN LF (lot # 0070808) and TBS (lot # (b) (4)) were supplied by Bavarian Nordic. ACAM2000 (lot # VV04-003-A) and ACAM2000 diluent (lot # (b) (4)) were obtained from the CDC (Atlanta, Georgia). The challenge virus, MPXV ((b) (4)), was obtained from (b) (4) .

Thirty-eight (38) specific pathogen-free cynomolgus macaques (19 males and 19 females) aged 2-5 years and weighing 2.61-3.53 kg were sourced from (b) (4) . After quarantine, animals were randomized into four groups (Table 10). Macaques in group 1 were inoculated with TBS on study Days 0 and 28. Group 2 (10 macaques) was vaccinated with MVA-BN (1×10^8 TCID₅₀ per macaque) on Days 0 and 28. Group 3 (10 macaques) was vaccinated with MVA-BN (1×10^8 TCID₅₀ per macaque) on Day 28, and group 4 (10 macaques) was vaccinated with ACAM2000 (2.5 - 12.5×10^5 PFU/macaque) on Day 28. Serum samples collected 7 days before vaccination, as well as on study Days 0, 7, 14, 21, 28, 35, 42, and 56, were tested IgG and neutralizing antibodies. Additional blood samples for antibody array were obtained on Day 0, 8, 28 and 56.

On Day 63, macaques were anesthetized and challenged with 5×10^6 PFU of MPXV and evaluated as described under Study BN-PRE-08-005 above. Blood samples for the assessment of viremia by real-time qPCR targeting the MPXV (b) (4) gene were collected on Days 63, 69, 72, 75, 78, 81, 84, 87, and 91.

Immunogenicity Results

All 10 macaques vaccinated with ACAM2000 developed a take with a mean lesion size of 0.47 cm² at peak (range, 0.55 to 0.89 cm²) on Day 35. Pre-vaccination serum samples from all groups and post-vaccination sera from macaques in the TBS group had no detectable IgG at any timepoint. In group 2, a 40% seroconversion rate (GMT of 8) was obtained seven days post-vaccination, and a 100% seroconversion was attained on Day 14 (GMT 582).

Table 13. Immunogenicity of MVA-BN Smallpox Vaccine in Study BN-PRE-09-003

Group	No. of Macaques	Vaccination (Schedule Day)	ELISA peak GMT (% seroconversion)	PRNT peak GMT (% seroconversion)
1	8	TBS (0, 28)	1 (0)	1 (0)
2	10	1×10^8 TCID ₅₀ (0, 28)	8686 (100)	741 (100)
3	10	1×10^8 TCID ₅₀ (28)	1106 (100)	5 (70)
4	10	2.5 - 12.5×10^5 PFU ACAM2000 (28)	387 (90)	10 (70)

Peak IgG titer was recorded on Day 35 (seven days after the second dose) with a mean GMT of 8686 (range, 4701 to 18501) (Table 13; adapted from Tables 4 & 5 and Appendices 1 & 2 of Amendment 1 to Study Report BN-PRE-09-003 in STN 125678/0). In group 3, 50% of macaques were seropositive seven days post-vaccination (Day 35; GMT of 14) and a peak GMT of 1106 (range, 200 to 8240) and 100% seroconversion was recorded on Day 42. Ninety percent (90%) of macaques vaccinated with ACAM2000 seroconverted (highest IgG GMT of 387 (range, 200 to 1112) on Day 56, with macaque #4645 failing to seroconvert. Neutralizing antibody was not detected in any pre-vaccination serum sample or group 1 serum samples at any timepoint. Fifty percent (50%) of group 2 antisera had neutralizing activity on

Day 7 (GMT = 3), and peak neutralizing of 741 (100% seroconversion) on Day 35 (Table 14; page 21 of Study Report in Amendment 1 to STN 125678/0). A peak PRNT GMT of 5 (70% seroconversion) was recorded on Day 42 for group 3, and a Day 56 GMT of 10 (70% seroconversion) for ACAM2000-vaccinated macaques. ACAM2000-vaccinated macaque # 4645 has no detectable neutralizing antibodies at any timepoint.

Table 14. Geometric Mean Titers of Neutralizing Antibodies in Study BN-PRE-09-003

	Group 1			Group 2			Group 3			Group 4		
	TBS			IMVAMUNE [®] 1x10 ⁸			IMVAMUNE [®] 1x10 ⁸ (once)			ACAM2000 [™]		
Day	% ¹	GMT ²	N ³	% ¹	GMT ²	N ³	% ¹	GMT ²	N ³	% ¹	GMT ²	N ³
-7	0	1	8	0	1	10	0	1	10	0	1	10
0	0	1	8	0	1	10	0	1	10	0	1	10
7	0	1	8	50	3	10	0	1	10	0	1	10
14	0	1	8	80	7	10	0	1	10	0	1	10
21	0	1	8	70	10	10	0	1	9	0	1	10
28	0	1	8	90	14	10	0	1	10	0	1	10
35	0	1	8	100	741	10	0	1	10	0	1	10
42	0	1	8	100	390	10	70	5	10	70	7	10
56	0	1	8	100	152	10	70	4	10	70	10	10

Animals in Group 1 to 3 were vaccinated (s.c.) with either TBS or IMVAMUNE[®] on Day 0 and 28. Animals in Group 4 were vaccinated with ACAM2000[™] (p.c.) on Day 28.

¹Seroconversion. ²Geometric mean titer. ³Number of animals.

Thus, in contrast to the previous studies (BN-PRE-08-004 and BN-PRE-08-005), both IgG and PRNT titers obtained in BN-PRE-09-003 were comparatively lower. Macaques treated with identical vaccination had variable antibody responses in the different studies (for example, BN-PRE-08-005 versus BN-PRE-09-003).

Results of MPXV Challenge (MPXV Morbidity and Survival)

No macaque, irrespective of treatment group, was recumbent or unresponsive or developed edema after MPXV infection. All macaques in the TBS group developed respiratory distress that included severe dyspnea and mild to moderate coughing. Moderate to severe anorexia, mild dehydration and mild to moderate weakness and depression were also recorded in the TBS group. Five group 1 macaques were euthanized on Day 71 and the remaining three on Day 72, all due to severity of MPXV disease. Gross necropsy of euthanized animals showed hard, discolored caudal lobes and pock lesions in some and pale liver was seen in two TBS macaques.

Mild dehydration was observed in all vaccinated animals, and mild to moderate dyspnea and anorexia were observed in most MVA-BN and ACAM2000 vaccinated macaques, but no sign of weakness or depression. Macaque #s 4643 (peak PRNT titer of 630 on Day 42) and 4646 (peak PRNT titer of 1110 on Day 42) in group 2 developed severe respiratory distress

(dyspnea with or without coughing) and were euthanized on Day 71. Hardened, pale discolored lungs and pale kidneys were observed in the vaccinated macaques that succumbed as well as in other vaccinated macaques at the end of the study. A macaque in group 3 that experienced anorexia and respiratory distress succumbed on Day 9 while under anesthesia. Thus, while all macaques inoculated with TBS succumbed to MPXV infection, majority of vaccinated animals, including MVA-BN cohort and ACAM2000 recipients, survived MPXV infection (Table 15; adapted from Table 2, Figure 2, and Appendix 8 of Study Report BN-PRE-09-003 in STN 125678/0). All macaques vaccinated with ACAM2000, including macaque #4645, survived.

Table 15. MPXV Morbidity and Mortality in Study BN-PRE-09-003

Group	Vaccination, (Schedule Day)	No. of macaques	Peak Mean Blood MPXV Load (genome copies/mL)	No of macaques without viremia at any timepoint	Survival (%)
1	TBS (0, 28)	8	1.0×10^7	0/8	0/8 (0)
2	MVA-BN 1×10^8 TCID ₅₀ (0, 28)	10	7.9×10^5	0/10	8/10 (80)
3	MVA-BN 1×10^8 TCID ₅₀ (28)	10	1.0×10^6	0/10	9/10 (90)
4	ACAM2000 2.5- 12.5×10^5 PFU (28)	10	7.9×10^4	3/10	10/10 (100)

The number of survivors in any of the vaccination groups was statistically significant when compared with the TBS group ($p = 0.001051$ versus group 2; $p = 0.000411$ versus group 3; and $p = 0.000023$ versus group 4 by applicant's analyses). Thus, similar to the results of the intravenous challenge studies, MVA-BN protected macaques from MPXV challenge in the intratracheal challenge model, though not conferring a 100% protection as ACAM2000.

Body Weight and Body Temperature

Most macaques experienced a transient weight loss between Day 66 to 78. Individual peak weight loss of between 8-14% of original body weight was recorded Day 71 to 72 among macaques in the TBS group (Figure 10; page 27 of Study Report BN-PRE-09-003 in STN 125678/0). The mean peak weight loss for macaques in groups 2, 3 and 4 on Day 72 were 5%, 2.5% and 3.5% respectively. All macaques in group 2 lost between 2-10% of weight from Day 66, and six of the eight (6/8) survivors had regained weight to baseline or higher levels by Day 87. The two group 2 macaques with the highest peak neutralizing antibody titers experienced prolonged weight loss. The two, macaque #s 4627 and 4630 had the highest peak titer of neutralizing antibodies (peak PRNT titer of 1140 and 1231, respectively), and the latter did not regain to baseline weight at the end of the study. In group 3, two macaques did not lose weight below baseline. The remaining macaques dropped below baseline from Day 66 and recovered to or above baseline from Day 78 to 87. Macaque # 4614 that succumbed on Day 9 (under anesthesia) lost 0.8% weight at the time it succumbed, and macaque #4636 with the highest peak PRNT titer in the group (peak PRNT titer = 37) did not regain weight to baseline throughout the monitoring period. Among ACAM2000 vaccinated macaque #4617 (peak PRNT titer = 66), #4632 (PRNT titer = 6), and #4645 (PRNT titer = 1) did not experience any weight loss. The remaining macaques experienced between 2% to 10% weight loss from Day 66 and

recovered to baseline or higher from Day 78 to 87. Two ACAM2000 vaccinated macaques (#s 4633 and 4637, both with a peak PRNT titer of 1) did not regain weight to baseline by the end of the study. Thus, the quantity of neutralizing antibodies did not appear to correlate with

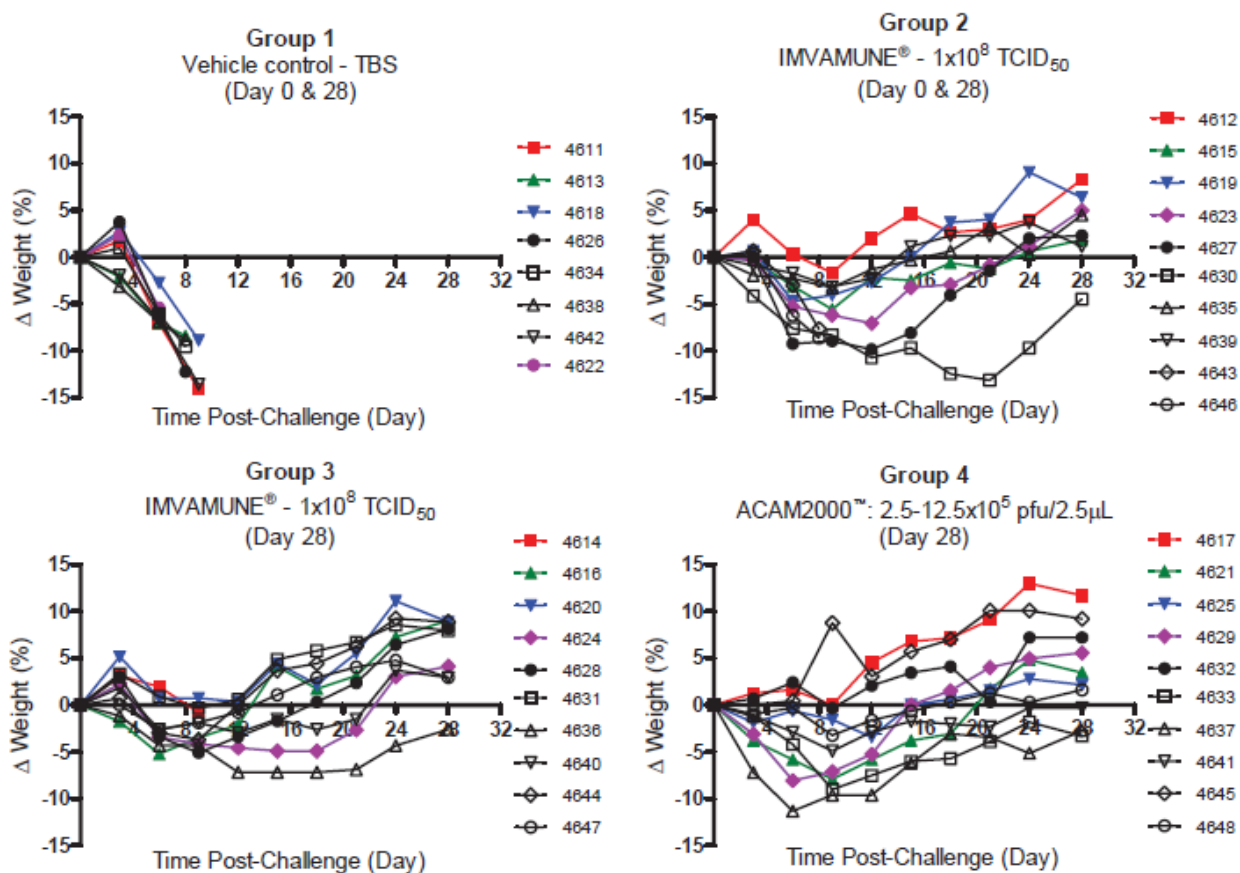


Figure 10. Individual Body Weight Changes After MPXV challenge in Study BN-PRE-09-003.

weight loss. By the applicant's analysis (Tukey test), there was a significant difference in weight loss between TBS group and vaccinated macaques (groups 2 to 4), but not between vaccinated macaque groups.

There were no apparent differences in body temperature changes between TBS-treated and vaccinated macaques from Day 63 to 72, but macaques that succumbed to MPXV infection experienced $\geq 3\%$ drop in body temperature before they were euthanized.

Pock Lesions

In the TBS group peak lesion counts in individual macaques were observed from Day 69 to 72 and ranged from 3 to 380. Five macaques in group 2 had no pock lesions at any timepoint, including # 4646 that succumbed to monkeypox. Except for macaque # 4630 that had a peak lesion count of 78 on Day 75, the remaining three survivors in group 2 had peak counts of 3, 3, and 10 lesions that resolved between Day 75 to 81. A macaque in group 3 had 1 lesion on Day 75 and a macaque in group 4 that previously had no lesion developed 40 lesions on Day 72. In both cases, the lesions were resolved by Day 78. The low numbers of pock lesions are

consistent with the intratracheal challenge model as observed in Study Report BN-PRE-08-005.

Blood Viral Load

Macaques in the TBS group had high viral loads that increased with time with a mean viral load of 10^7 genome copies/mL on Day 69 before the first set of five was euthanized. Further increases in individual viral load was recorded in the second set of three that was euthanized on Day 72. Peak viremia (7.9×10^5 genome copies/mL) was recorded in group 2 on Day 69 (Table 15). All group 2 macaques had viremia at different timepoints (Figure 11; page 34 of Study Report BN-PRE-09-003 in STN 125678/0). Viremia persisted through Day 81 in 3 of 8 survivors but were all cleared by Day 87. In group 3, all macaques had viremia at different timepoints, with a peak of 10^6 genome copies/mL from Day 69 to 72.

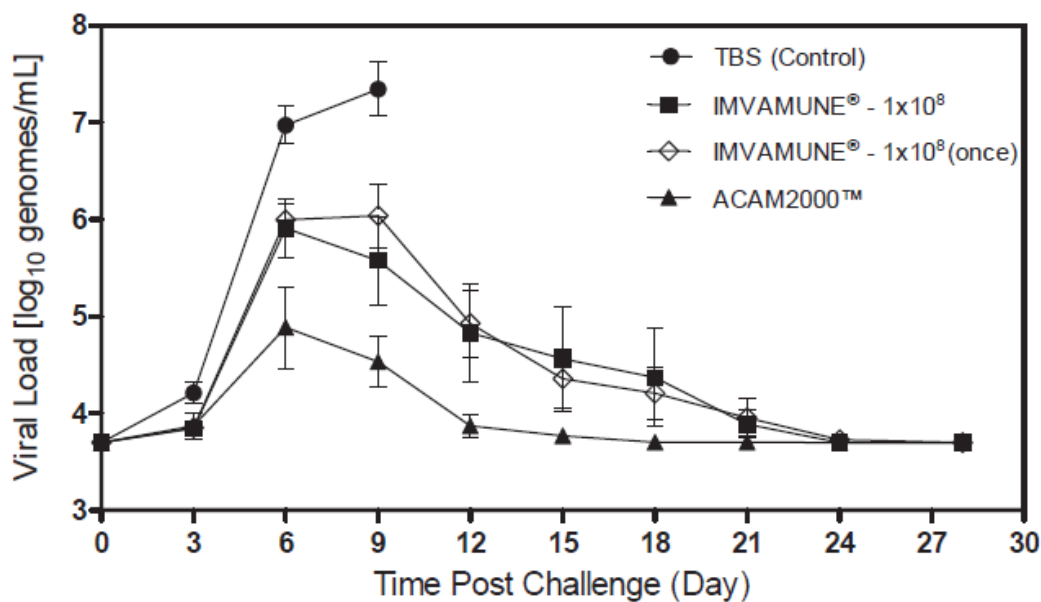


Figure 11. Mean Blood Viral Load (\log_{10}) Following MPXV Challenge in Study BN-PRE-09-003

Viremia was resolved in majority of surviving macaques (6/9) by Day 84 and in all group 3 macaques by Day 91. Transient viremia was observed in 7/10 ACAM2000 vaccinated macaques at different time points between Day 66 to 75, with majority (6/7) cleared by Day 78. The mean viral load at peak was 7.9×10^4 genome copies/mL on Day 69. One macaque had detectable MPXV genome through Day 87. The remaining 3 macaques in group 4 had below LOD levels of MPXV genome.

White Blood Cell Count

Increase in white blood cell (WBC) count was observed in all macaques after MPXV challenge but was more prominent in the TBS group. Group 1 macaque #s 4613, 4618 and 4638 had a 3 to 4-fold rise in WBC at the time of euthanasia. Group 2 macaque #4630 and group 4 macaque # 4645 that had the highest WBC count on Day 91 had the highest peak viral load in their respective group.

Review comment: Consistent with the results of the intratracheal challenge described in Study Report BN-PRE-08-005, vaccination with MVA-BN elicited antibody responses and conferred protection on macaques following intratracheal challenge with MPXV.

Study BN-PRE-09-004 (NHP/MPXV Intratracheal Challenge)

Title

Comparison of the Immunogenicity and Protective Efficacy of IMVAMUNE® and ACAM2000™ in the Intratracheal Monkeypox Challenge Model in Cynomolgus Monkeys

Study Design Summary

Study BN-PRE-09-004 was conducted under study number SR09-004F at SRI. The study had similar overall objectives as BN-PRE-08-005 and BN-PRE-09-003 in evaluating the immunogenicity and protective efficacy of MVA-BN in comparison with ACAM2000 in the intratracheal challenge model. However, the study was designed to compare a prime/boost regime of MVA-BN with ACAM2000, and contained two identical iterations with respect to vaccination, allowing for the use of two challenge doses (high and low) of MPXV in the challenge phase (Table 16; adapted from Table 2 of Study Report BN-PRE-09-004 in Amendment 23 to STN 125678). Also included as an objective was the identification of a potential humoral immune correlate of protection. However, in Amendment 1 to the study report, the applicant indicated that immunogenicity data (ELISA and PRNT) would not be provided in the final report.

Table 16. Design of Study BN-PRE-09-004

Group	No. of macaques	Vaccination	Dose	Route	Vaccination schedule (study day)	MPXV challenge on day 63 (PFU)
1	6	TBS	N/A	SC	0, 28	5x10 ⁶
2	6	MVA-BN	1x10 ⁸ TCID ₅₀	SC	0, 28	5x10 ⁶
3	6	ACAM2000	2.5-12.5x10 ⁵ PFU	PC	28	5x10 ⁶
4	6	TBS	N/A	SC	0, 28	1x10 ⁶
5	6	MVA-BN	1x10 ⁸ TCID ₅₀	SC	0, 28	1x10 ⁶
6	6	ACAM2000	2.5-12.5x10 ⁵ PFU	PC	28	1x10 ⁶

N/A, Not Applicable; SC, subcutaneous

Clinical-grade MVA-BN LF (lot # 0050808) and TBS (lot # (b) (4)) were supplied by Bavarian Nordic. ACAM2000 (lot # VV04-003-A) and ACAM2000 diluent (lot # (b) (4)) were obtained from the CDC (Atlanta, Georgia); and MPXV ((b) (4)) was obtained from (b) (4).

Thirty-six (36) specific pathogen-free cynomolgus macaques (18 males and 18 females) aged 2-5 years and weighing 2.55-6.0 kg were sourced from (b) (4).

(b) (4). After quarantine, animals were randomized into six groups, each containing six macaques (Table 16). Group 1 and 4 were inoculated with TBS on study Days 0 and 28; groups 2 and 5 were vaccinated with MVA-BN (1×10^8 TCID₅₀ per macaque) on Days 0 and 28; and groups 3 and 6 were vaccinated with ACAM2000 ($2.5\text{--}12.5 \times 10^5$ PFU/macaque) on Day 28. Prior to MPXV challenge, a group 1 macaque developed rectal prolapse and was excluded from the study (euthanized). On Day 63, each macaque was anesthetized by intramuscular injection of ketamine (10-30 mg/kg), xylazine (0.35 mg/kg) and atropine (0.04 mg/kg) and challenged by delivery of 5×10^6 PFU (groups 1 to 3) or 1×10^6 PFU (groups 4 to 6) of MPXV into the mid-to-distal trachea using a flexible bronchofiberscope. Throat swab samples were collected on Days 63, 66, 69, 72, 75, 78, 81, 84, 87, and 91, for the assessment of MPXV shedding.

Blood samples for the assessment of viremia by real-time qPCR targeting the MPXV (b) (4) gene were collected on Days 63, 69, 72, 75, 78, 81, 84, 87, and 91. Following MPXV challenge, animals were evaluated as described in Study Report BN-PRE-08-004.

Immunogenicity Results

All macaques vaccinated with ACAM2000 developed a vaccine take, with mean peak (Day 35) take lesion sizes of 0.564 cm^2 and 0.293 cm^2 , respectively. Take lesions were completely resolved by Day 56, except in macaque #s 4690 and 4696 in group 6 that had Day 56 lesion sizes of 0.296 cm^2 and 0.046 cm^2 , respectively. No macaque in other treatment groups developed a vaccine take. Although the study protocol indicated that serum samples for immunogenicity evaluation were collected 21 days before vaccination, as well as on study Days 0, 7, 14, 21, 28, 35, 42, and 56, but as stated above, there were no additional immunogenicity data (ELISA and PRNT) from study BN-PRE-09-004 submitted in STN 125678/0.

Results of MPXV Challenge (MPXV Morbidity and Survival)

Similar clinical symptoms were precipitated by MPXV at both high (5×10^6 PFU) and low (1×10^6 PFU) challenge doses. Macaques in groups 1 & 4 developed respiratory distress (moderate-to-severe dyspnea and coughing), moderate-to-severe depression, and anorexia, as well as moderate-to-severe dehydration. All five (5/5) macaques in group 1 were euthanized from Day 71 to 75 due to disease severity (Table 17; adapted from Appendices, 8, 9 & 10 of Study Report BN-PRE-09-004 in Amendment 23 to STN 125678/0).

Among vaccinated animals, clinical symptoms of MPXV infection, including dehydration, dyspnea, anorexia and coughing were generally similar to those in the TBS groups but were relatively milder. A group 2 macaque with no clinical signs of depression, weakness or nasal discharge, had the highest blood viral load of 2.2×10^8 genome copies/mL at the Day 72 timepoint and was found dead on Day 73. Thus, 5/6 (83%) of macaques vaccinated with MVA-BN prime/boost as proposed in humans survived high-dose MPXV challenge. A 100% survival rate was recorded for macaques vaccinated with ACAM2000 and challenged with a high dose of MPXV.

In the low-dose MPXV challenge cohort, 4/6 macaques in the TBS group (group 4) were euthanized from Day 72 to 78 due to disease severity, with two (33%) macaques surviving. The two survivors experienced moderate-to-severe dehydration, moderate respiratory distress (dyspnea, cough), mild-to-moderate depression and moderate anorexia, a 2-4% increase in body temperature on Days 75 and 78, viremia that persisted through Day 84 (1 macaque) and till Day 91 in the second survivor. Group 4 survivors experienced peak weight loss of 10% and

14%, and shed MPXV DNA in throat samples through the end of study (mean Day 91 throat swab load = 9×10^5 genome copies/mL). All vaccinated macaques (groups 5 and 6) in the low-dose challenge cohort survived.

In the applicant's analysis of survival in the high-dose challenge cohort, the difference in survival rate between the TBS group and either of the vaccinated groups (MVA-BN or ACAM2000) was statistically significant ($p = 0.015$ MVA-BN vs TBS; and $p = 0.0022$ for ACAM2000 vs TBS) but the difference between groups 2 and 3 was not significant ($p = 1$). However, in the low-dose MPXV challenge cohort, no statistically significant difference was found among the three treatment groups ($p = 0.0606$ for group 5 versus group 4; $p = 0.0606$ for group 6 versus group 4; and $p = 0.0606$ for group 5 versus group 6).

Table 17. Morbidity and Mortality Following MPXV Challenge in Study BN-PRE-09-004.

Group	No. of macaques	Vaccination (Schedule Day)	MPXV Challenge on Day 63 (PFU)	Peak Mean Total Body Lesion Count	Peak Mean Blood MPXV Load (genome copies/mL)	No of macaques without viremia at any timepoint	Peak Mean Throat MPXV Shedding (genome copies/mL)	Survival (%)
1	6*	TBS (0, 28)	5×10^6	322	4.4×10^6	0/5	2.6×10^7	0/5* (0)
2	6	MVA-BN, 1×10^8 TCID ₅₀ (0, 28)	5×10^6	0	6.0×10^7	0/6	4.7×10^7	5/6 (83)
3	6	ACAM2000, 2.5 - 12.5×10^5 PFU (28)	5×10^6	0	4.7×10^5	2/6	4.3×10^5	6/6 (100)
4	6	TBS (0, 28)	1×10^6	219	2.1×10^7	0/6	2.9×10^8	4/6 (67)
5	6	MVA-BN, 1×10^8 TCID ₅₀ (0, 28)	1×10^6	2**	4.3×10^6	1/6	8.8×10^6	6/6 (100)
6	6	ACAM2000, 2.5 - 12.5×10^5 PFU (28)	1×10^6	0	1.0×10^5	3/6	1.3×10^5	6/6 (100)

*A macaque assigned to group 1 was euthanized before MPXV challenge due to rectal prolapse.

**This value was due to a macaque in the group having 14 lesions on Day 78.

Body Weight and Body Temperature

There were no apparent differences in body weight changes with respect to MPXV challenge dose. Individual weight changes of between 5-14% were recorded among macaques in the TBS/high-dose challenge group (group 1) and 10-14% in the TBS/low-dose challenge group (group 4). Macaques in the MVA-BN treatment groups (groups 2 and 5) lost more weight than those vaccinated with ACAM2000 (groups 3 and 6) (Figure 12 A & B; page 25 of Study Report BN-PRE-09-004 in STN 125678/23). Among MVA-BN vaccinated macaques, weight loss

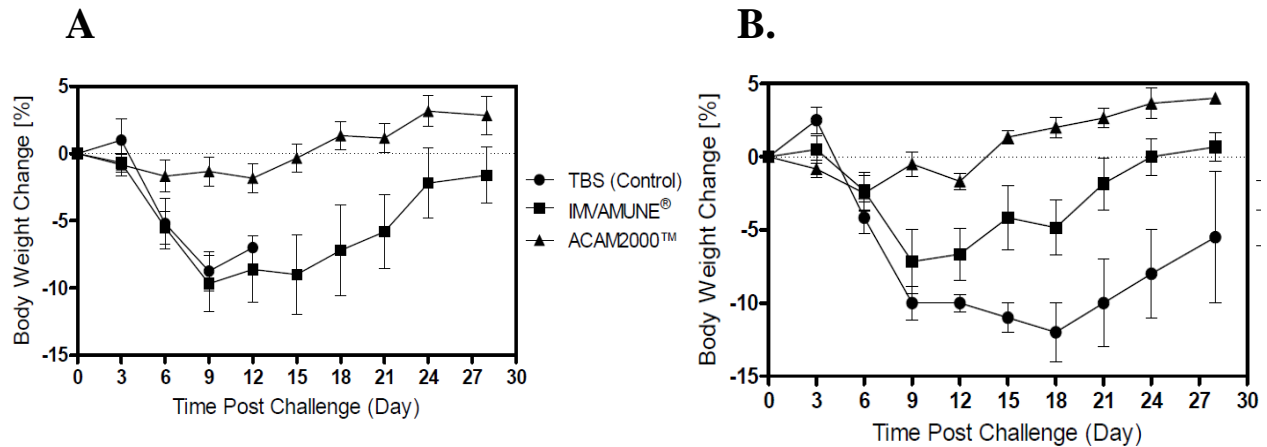


Figure 12. Mean Body Weight Changes Following High-dose (5×10^6 PFU (A)) and Low-dose (1×10^6 PFU (B)) MPXV Challenge in Study BN-PRE-09-004.

returned to baseline by Day 87 in the low-dose challenge group but persisted through the end of the study in the high-dose challenge group. Weight loss in the ACAM2000 treatment group was transient and all macaques (in both high-dose and low-dose challenge groups) had returned to or were above baseline by Day 78.

None of the treatment groups in the study had persistent fever after MPXV infection. Two of five (2/5) macaques that succumbed in the TBS group experienced 5% (macaque #4712) and 8% (macaque #4700) decrease in body temperature at the time of euthanasia on Day 75 and Day 73, respectively. Except for macaque # 4690 in ACAM2000/high-dose challenge group that had a 6% decrease in body temperature on Day 66 (and back to baseline on Day 69), all vaccinated macaques had $\leq 3\%$ change in body temperature.

White Blood Cell Counts

After MPXV challenge, WBC counts increased in vaccinated and unvaccinated macaques irrespectively of vaccine type but appeared to be higher in the high-dose challenge cohort (groups 1 to 3). The highest peak levels of WBC count in blood samples were recorded in the unvaccinated (TBS) group 1 (range, $11.74 - 31.52$ m/mm³) and group 4 (range, $13.74 - 23.92$ m/mm³). Among vaccinated macaques, a range of $12.40-23.52$ m/mm³ and a range of $11.18-24.18$ m/mm³ were the peaks for the individual macaque in groups 2 and 3, respectively. Peak ranges of $11.82-23.72$ m/mm³ and $9.52-13.84$ m/mm³ were recorded in the MVA-BN and ACAM2000 vaccinated groups of the low-dose challenge cohort, respectively.

Although higher WBC counts were recorded in unvaccinated macaques, there was no clear difference in WBC counts among individual macaques irrespectively of vaccination treatment.

Pock Lesions

A macaque in the TBS/high-dose challenge group did not develop pock lesions at any timepoint but succumbed on Day 72. The other four macaques developed pocks beginning from Day 69, with a mean of 322 (range 79 to 700) at peak on Day 72 (Table 17). The last group 1 macaque to be euthanized on Day 75 had 469 pock lesions at euthanasia. Except for a macaque in group 2 that had a single pock lesion on Day 72, all vaccinated macaques (MVA-BN and ACAM2000) had no pock lesions at any timepoint. In the low-dose MPXV challenge cohort, pock lesions were recorded from Day 69 in the TBS group (group 4) and all macaques

in the group had pock lesions at different timepoints. A peak lesion count of 219 (range, 47 to 700) was recorded on Day 72. Pock lesions persisted in the two group 4 survivors through Day 87, and one of them had 47 lesions on the last day of the study. In the MVA-BN/low-dose treatment group, a macaque had a single lesion on Day 69 and a second macaque had 14 lesions on Day 78. All other macaques in the group as well as the ACAM2000 treatment group had no pock lesion at any timepoint.

Blood Viral Load

All macaques in the TBS/high-dose challenge group had viremia beginning from Day 66, before they were euthanized from Day 69 to 72. A peak blood viral load of 4.4×10^6 genome copies/mL was recorded on Day 69 and viral load increased in the four macaques that were euthanized from Day 72-73 (Figure 13 (A); page 29 of Study Report BN-PRE-09-004 in Amendment 23 to STN 125678). Similarly, all macaques in the MVA-BN vaccinated/high-dose MPXV challenge group had viremia at some timepoint. In 4/5 surviving macaques in the group, viremia persisted through Day 81 but was fully cleared by Day 87. Peak viremia occurred on Day 72 with a mean of 6.0×10^7 genome copies/mL (range, 2.1×10^6 to 2.2×10^8 copies/mL). A macaque in the group had below-detection level on Day 72 but experienced transient viremia on Days 69 and 78. In group 3 (ACAM2000/high-dose challenge group), 4/6 had transient viremia from Day 69 to Day 75, two of which had low levels of MPXV genome from Day 78 to 84. Mean viral load at peak on Day 69 was 4.7×10^5 genome copies/mL. Viremia was resolved in all ACAM2000-vaccinated macaques by Day 84.

In the low-dose MPXV challenge cohort, all six unvaccinated macaques (TBS; group 4) had high viral load from Day 69 to 72 peaking at 2.1×10^7 (range 3.5×10^6 to 5.2×10^7) genome copies/mL on Day 72. Viremia persisted in the two survivors through Day 84 and was not resolved in one of them at the end of the study (Figure 13 (B); page 31 of Study Report BN-PRE-09-004 in Amendment 23 to STN 125678/0).

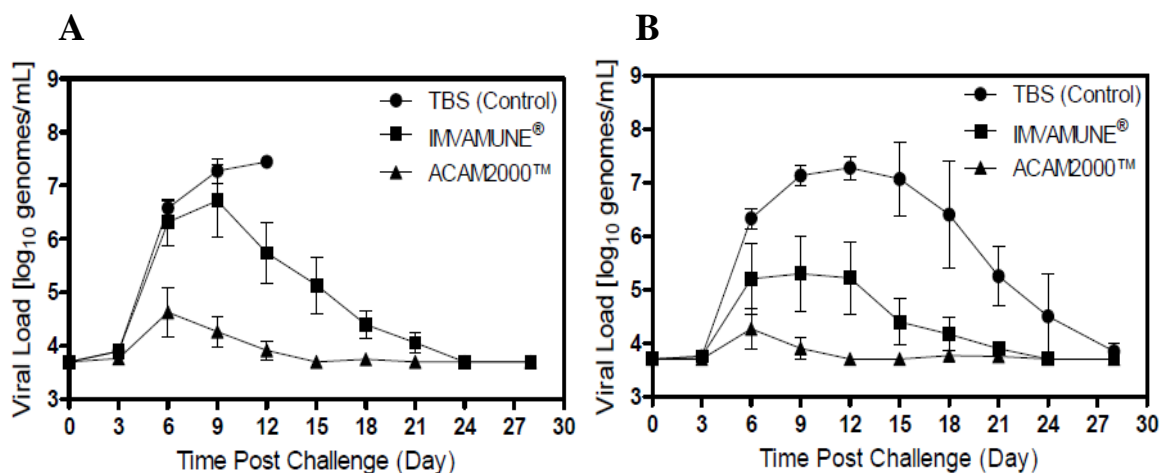


Figure 13. Mean Viral Load in Blood Following High-dose (5×10^6 PFU) (A) and Low-dose (1×10^6 PFU) (B) MPXV Challenge in Study BN-PRE-09-004.

Macaque #4704 in the MVA-BN vaccinated/low-dose challenge group (group 5) had no viremia at any timepoint and macaque #4698 in the same group had transient viremia on Day 84 (1.5×10^4 copies/mL). Peak viremia was recorded on Day 72 with a mean of 4.3×10^6 copies/mL.

Macaques vaccinated with ACAM2000 prior to low-dose MPXV challenge had the least viral load. Three of six (3/6) in the group had no detectable MPXV genome and two others had transient viremia with MPXV genome detected at one or three timepoints. Macaque #4699 in the group had the highest viral load of 4.6×10^5 genome copies/mL on Day 69 and primarily accounted for the group's mean peak of 1×10^5 genome copies/mL at this timepoint. This macaque had low levels of MPXV genome detected on Days 72, 81, and 84 and was cleared by Day 87.

Throat Virus Shedding

Among macaques in the high-dose challenge cohort virus shedding in throat was evident in group 1 macaques on Day 66 reaching 2.6×10^7 genome copies/mL (Figure 14 (A); page 30 of Study Report BN-PRE-09-004 in Amendment 23 to STN 125678) before the first macaque was euthanized on Day 8. Viral shedding increased in the remaining macaque reaching 3.7×10^7 genome copies/mL before three more macaques were euthanized. The last macaque in the group had a virus load of 2.4×10^8 genome copies/mL at the time of euthanasia on Day 75. Four of six (4/6) macaques in group 2 were shedding MPXV on Day 66, and by Day 69, all in the group were shedding virus. Prior to one macaque succumbing on Day 73, the mean virus shedding for the group was 4. genome copies/mL on Day 72. By Day 75, mean virus shedding for the remaining 5 macaques had increased to 1×10^8 genome copies/mL. Except for one macaque that shed virus through the end of the study, virus shedding was below LOD by Day 84. With the exception of macaque #4690, all macaques vaccinated with ACAM2000 and challenged with high dose MPXV (group 3) shed virus in at least one timepoint but virus shedding was not apparent until Day 69 (3/6 macaques) when virus shedding also peaked (mean of 4.3×10^5 genome copies/mL). On Day 78, all group 3 macaques were below LOD. However, on Day 81, two macaques that previously had no detectable virus genome had a transient virus shedding that was cleared three days later.

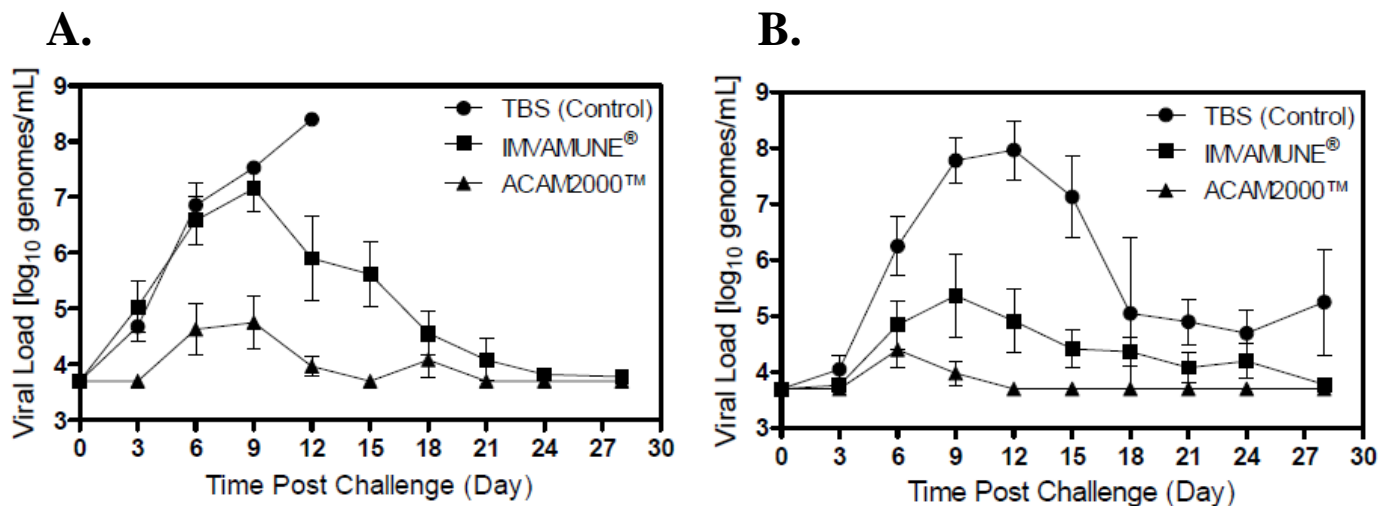


Figure 14. Mean Viral Load in Throat Swabs Following High-dose (5×10^6 PFU) (A) and Low-dose (1×10^6 PFU) (B) MPXV Challenge in Study BN-PRE-09-004.

In the low-dose MPXV challenge cohort, 5/6 macaques in the TBS group (group 4) were shedding virus by Day 69. By Day 72, all macaques in the group were shedding MPXV at peak, with a group mean of 2.9×10^8 genome copies/mL (Figure 14 (B); page 31 of Study Report BN-PRE-09-004 in Amendment 23 to STN 125678). The two group 4 survivors shed

MPXV at all timepoints, with a mean of 9×10^5 genome copies/mL at the termination of study on Day 91.

All macaques in the MVA-BN/low-dose challenge group shed virus at least once at the different timepoints. A mean shedding of 8.8×10^6 genome copies/mL was recorded at peak on Day 72. Three macaques remained virus-shedding through Day 87 and macaque #4710 did not clear virus by Day 91. Two macaques vaccinated with ACAM2000 were below LOD at all timepoints. The remaining four macaques had transient MPXV shedding on Day 69 with a mean (peak) shedding of 1.3×10^5 genome copies/mL. Half (2/4) of the virus-shedding macaques had resolved by Day 72 and all group 6 macaques were below LOD from Day 75.

Review Comment: Morbidity and survival data following MPXV challenge of vaccinated and control animals in study BN-PRE-09-004 indicated that MVA-BN did not protect all macaques from a high-dose challenge as did ACAM2000. Nonetheless, data from this study corroborated the efficacy of MVA-BN in protecting against MPXV morbidity and mortality in the intratracheal challenge model as previously demonstrated in studies BN-PRE-08-005 and BN-PRE-09-003.

Study BN-PRE-11-021 (NHP/MPXV Aerosol Challenge)

Title

Determination of the Optimal Dose of Monkeypox for the Aerosol Challenge Model in Cynomolgus Macaques and Evaluation of Efficacy and Immunogenicity of IMVAMUNE

Study Design Summary

Study BN-PRE-11-021 was conducted under study number 1216-100005125 at BBRC. The study was designed to evaluate the immunogenicity and protective efficacy (morbidity and mortality) of the LF (b) (4) formulations of MVA-BN in a respiratory MPXV aerosol challenge model. The study design contained two iterations with respect to vaccination, allowing for the use of two challenge doses, a low dose (1×10^5 PFU) and a high dose (3×10^5 PFU) of MPXV in the challenge phase (Table 18; adapted from page 16 of Study Report BN-PRE-11-021 in Amendment 28 to STN 125678).

Table 18. Design of Study BN-Pre-11-021

Group	No. of macaques	Vaccination	Vaccine Dose (TCID ₅₀)	Route	Vaccination schedule (study day)	MPXV challenge on day 63 (PFU)
1	6	TBS	N/A	SC	0, 28	1×10^5
2	6	MVA-BN LF	1×10^8	SC	0, 28	1×10^5
3	6	MVA-BN (b) (4)	1×10^8	SC	0, 28	1×10^5
4	6	TBS	N/A	SC	0, 28	3×10^5
5	6	MVA-BN LF	1×10^8	SC	0, 28	3×10^5
6	5	MVA-BN (b) (4)	1×10^8	SC	0, 28	3×10^5

N/A, Not Applicable; TCID₅₀, 50% Tissue Culture Infectious Dose; SC, Subcutaneous.

Test articles, including TBS (Lot # (b) (4)), MVA-BN-LF (Lot # M00181) and MVA-BN-^{(b) (4)} (Lot # (b) (4)) were supplied by the applicant. MPXV ((b) (4)) was obtained from (b) (4).

Thirty-five (17 males; 18 female) healthy, specific pathogen-free macaques weighing 2.6-4.1 kg (age range not stated) sourced from (b) (4) were quarantined and acclimatized at BBRC. Macaques were weighed and randomized by age and sex into six treatment groups (Table 18). Due to limitation in the number of macaques that can be exposed to aerosol per day, each group was further randomly subdivided into two challenge cohorts containing equal numbers of macaques (except group 6). In all procedures (first and second vaccinations, and MPXV challenge) the second set of subgroups (Challenge Day B, CDB group) was treated a day after the first set of subgroups (Challenge Day A, CDA group).

Seven days before vaccination, pre-vaccination blood samples for ELISA, PRNT and PBMC isolation were collected. On study Day 0, pre-vaccination blood samples for PBMC isolation, ELISA and PRNT were collected from macaques, and the first set of macaques in the TBS groups (groups 1 & 4) were inoculated with 0.5 mL TBS. Similarly, each macaque in the first set in groups 2 and 5 was inoculated with 1×10^8 TCID₅₀ of MVA-BN LF, and each macaque in groups 3 and 6 was inoculated with 1×10^8 TCID₅₀ of MVA-BN ^{(b) (4)}. The second set of macaques in all groups was treated as their group peers the following day. All inoculations were delivered via the SC route. Post-vaccination blood samples for ELISA and PRNT were collected on study Days 7, 14, 21, and 28 after vaccination. On study Day 28, all macaques in the first set received a second (boost) treatment identical to the treatment on Day 0, and macaques in the second set received the boost treatment a day after. Post-boost blood samples were collected on Days 35, 42, and 56 for ELISA and PRNT. Additional blood samples for PBMC isolation were collected on Day 56.

On Day 63, the first set of macaques (CDA cohort) was anesthetized by intramuscular injection of Telazol (3 mg/kg) and challenged with a target dose of 1×10^5 PFU or 3×10^5 PFU of MPXV by a single-animal head-only exposure to aerosolized MPXV. Aerosolized MPXV was generated using a modified (b) (4) nebulizer that produced aerosols of 1-2 μ m in diameter. The second set of macaques (CDA cohort) were similarly infected with 1×10^5 PFU or 3×10^5 PFU of aerosolized MPXV a day after. The aerosol exposure system consisted of the exposure chamber, chamber samplers, an aerosol particle size analyzer, temperature and humidity monitoring, mass flow meters (MFM) and mass flow controllers (MFC) to monitor the aerosol flows. Aerosol samples from the exposure chamber was obtained by impaction on a (b) (4) filter for the quantitation of aerosolized MPXV, and the liquid in the nebulizer was tested by plaque assay for MPXV concentration. MPXV titer from the (b) (4) filter sample was used to determine the actual exposure dose for each macaque. The inhaled dose of MPXV was determined by real-time plethysmography and the concentration of MPXV in air samples from the exposure system was determined.

Blood samples intended for ELISA and PRNT were collected on Days 63, 66, 69, 75, 81, 87, and 91. Blood samples and throat swabs for the assessment of viremia and virus shedding were collected on Day 63 and every 3 days through day 87 and day 91. After MPXV challenge, macaques were observed for behavior, physical appearance, feces output, eating behavior, and movement/activity. Monkeypox lesions were on the arms, legs, ventral torso, head and dorsal torso were counted. Macaques were weighed and body temperatures measured on these days. Clinical assessment scores of 1, 2, and 3 were assigned for mild, moderate and

severe observations, respectively. An animal adjudged moribund after clinical evaluation was euthanized prior to the scheduled day. The euthanasia judge was familiar with the set of euthanasia criteria but was blinded to the treatment group each animal evaluated belonged (Amendment 5 to BBRC Protocol 1216 for study 1216-1000005125). For the assessment of viremia and virus shedding, viral load was evaluated by qPCR as described under study BN-Pre-12-003. At euthanasia or when a macaque was found dead, gross necropsy was performed and various tissues and organs were collected for examination.

Immunogenicity Results

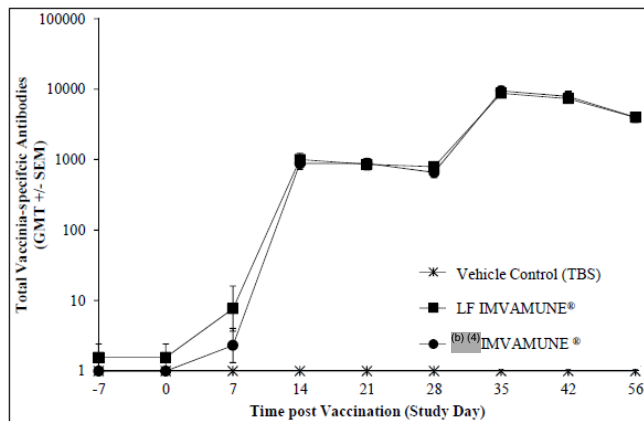
Pre-vaccination serum samples had no vaccinia-specific IgG except in a group 5 macaque (# A08834) that had a pre-vaccination ELISA titer of 200 (but seronegative in PRNT). None of the macaques treated with TBS (groups 1 & 4) seroconverted for binding and neutralizing antibodies. All MVA-BN vaccinated macaques seroconverted both by ELISA and PRNT, irrespective of which vaccine formulation they received. Both ELISA and PRNT GMTs peaked on Day 35, except for group 6 where the PRNT GMT peaked on Day 56. Peak ELISA GMTs were 7988 (range, 4843 to 12782); 10701 (range, 3984 to 25373); 9570 (range, 4680 to 12446); and 8137 (range, 5816 to 18564) for vaccinated groups 2, 3, 5 and 6, respectively (Appendix 1; pages 757 to 765 of Study Report BN-PRE-11-021 in Amendment 28 to STN 125678). Macaque # A08834 that was seropositive pre-vaccination had the highest IgG titer of 422 (and a PRNT titer of 3) at the seven days post-vaccination timepoint and maintained this status at other measurement timepoints through Day 28 (titer of 3012), suggesting that the antibody response after the first vaccination was anamnestic. Thus, macaque A08834 may have had a prior exposure to an orthopoxvirus. However, after the second vaccine dose on Day 28, vaccinia-specific

Table 19. Immunogenicity of MVA-BN Smallpox Vaccine in Study BN-PRE-11-021

Group	No. of Macaques	Vaccination, TCID ₅₀ (Schedule Day)	Peak ELISA GMT (seroconversion)	Peak PRNT GMT (seroconversion)
1	6	TBS (0, 28)	1 (0%)	1 (0%)
2	6	MVA-BN LF, 1x10 ⁸ (0, 28)	7988 (100%)	379 (100%)
3	6	MVA-BN (b) (4), 1x10 ⁸ (0, 28)	10701 (100%)	400 (100%)
4	6	TBS (0, 28)	1 (0)	1 (0%)
5	6	MVA-BN LF, 1x10 ⁸ (0, 28)	9570 (100)	506 (100%)
6	5	MVA-BN (b) (4), 1x10 ⁸ (0, 28)	8137 (100)	338 (100%)

antibody response in macaque A08834 (titer of 8831) was within the range for group 5, and was below the group GMT at peak on Day 35, as well as at other subsequent timepoints. Similar to the results obtained in the ELISA, PRNT GMTs at peak were 379 (range, 104 to 2175); 400 (range, 78 to 1361); 506 (range, 208 to 1157); and 338 (range, 135 to 889; Day 56) for vaccinated groups 2, 3, 5 and 6, respectively (Appendix 2; pages 766 to 774 of Study Report BN-PRE-11-021 in Amendment 28 to STN 125678). Combining data for groups that received identical vaccination indicates similar trends in both ELISA and PRNT GMTs (Figure 15; pages 775 and 776 of Study Report BN-PRE-11-021 in Amendment 28 to STN 125678). The applicant's analyses indicate that there were no statistical differences in ELISA and PRNT GMTs between groups vaccinated with LF MVA-BN and those vaccinated with (b) (4) MVA-BN.

ELISA GMTs



PRNT GMTs

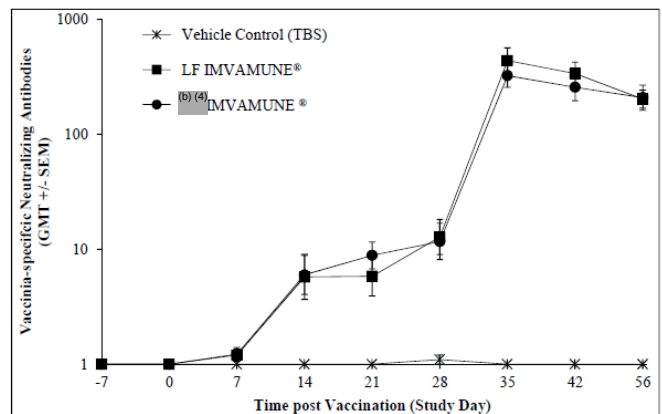


Figure 15. Immunogenicity of MVA-BN LF and MVA-BN (b) (4) in Study BN-PRE-11-021

Results of MPXV Challenge (MPXV Morbidity and Survival)

Testing of sampled aerosol during the aerosol exposure process on both CDA and CDB indicated that over the two challenge days, the mean actual delivered dose for the targeted low dose of 1×10^5 PFU was 7.26×10^4 PFU (range, 4.51×10^4 to 1.25×10^5 PFU) (page 154 of Study Report BN-PRE-11-021 in Amendment 28 to STN 125678). In the high-dose challenge cohort, the mean actual delivered dose over the two challenge days was 1.76×10^5 PFU (range, 1.22×10^5 to 2.24×10^5 PFU). The inability to attain the target challenge doses of 1×10^5 PFU and 3×10^5 PFU for the low-dose and high-dose challenge may be due in part to the titer of the virus as BBRC determined the titer of MPXV NR-21738 lot 59808225 to be 8.62×10^7 PFU/mL, albeit the certificate of analysis from (b) (4) indicated a titer of 3.7×10^8 PFU/mL (page 699 of Study Report BN-PRE-11-021 in Amendment 28 to STN 125678). Macaque #A11191 in group 5 was reported to have pulled its head out of the aerosol exposure chamber, making it impossible to attain the target total accumulated tidal volume. This should, theoretically, cause a reduction in the actual dose of MPXV the macaque inhaled. However, the actual dose of MPXV inhaled by macaque #A11191 was determined to be 1.95×10^5 PFU, higher than the mean actual delivered dose for macaques in the 3×10^5 PFU challenge cohort.

Following aerosol challenge with MPXV, similar clinical symptoms were observed in the TBS groups (groups 1 and 4), including dehydration, moderate to severe hunched posture, respiratory distress (coughing, open-mouth breathing, and labored breathing), and moderate to severe inappetence from Day 69 to 75. Two group 1 macaques that experienced severe dyspnea succumbed to infection. Nasal and ocular discharges in some macaques, and sneezing, lack of stooling and vomiting were infrequent. Group 1 macaque # A10783 also experienced swelling of the eyelids. All macaques vaccinated with MVA-BN, irrespective of the formulation, survived (Table 20; adapted from: pages 575 to 578 of Appendix G; and pages 624 to 625, and 633 to 634 of Appendix J of Study BN-PRE-11-021 in Amendment 28 to STN 125678). However, 67% (4/6) of macaques in the TBS/low-dose challenge also survived, albeit with moderate-to-severe MPXV morbidity (Figure 16, page 524 of Study Report BN-PRE-11-021 in STN 125678/0). The two group 1 macaques that succumbed were euthanized on Days

73 and 75. The three female macaques in group 1 experienced the highest total clinical scores of 10 to 15,

Table 20. MPXV Morbidity and Mortality in Study BN-PRE-11-021

Group	No. of macaques	Vaccination (Schedule Day)	Aerosol MPXV Challenge on Day 63 (PFU)	Peak Mean Total Body Lesion Count	Macaques without pock lesions	Peak Mean Blood MPXV Load (genome copies/mL)	No of macaques without viremia at any timepoint	Peak Mean Throat MPXV Shedding (genome copies/mL)	Survival (%)
1	6	TBS (0, 28)	1×10^5	12.2	1	1.4×10^7	0/6	4.4×10^5	4/6 (67)
2	6	MVA-BN LF, 1×10^8 TCID ₅₀ (0, 28)	1×10^5	1.5	4	6.9×10^2	2/6	2.8×10^4	6/6 (100)
3	6	MVA-BN (b) (4), 1×10^8 TCID ₅₀ (0, 28)	1×10^5	0	6	1.2×10^4	0/6	3.9×10^4	6/6 (100)
4	6	TBS (0, 28)	3×10^5	10	3	1.3×10^7	0/6	4.5×10^6	1/6 (17)
5	6	MVA-BN LF, 1×10^8 TCID ₅₀ (0, 28)	3×10^5	0.17	5	9.5×10^3	0/6	6.3×10^4	6/6 (100)
6	5	MVA-BN (b) (4), 1×10^8 TCID ₅₀ (0, 28)	3×10^5	0.2	4	1.9×10^3	0/5	5.4×10^4	5/5 (100)

and two of them succumbed to MPXV infection; all three male macaques in group 1 survived with moderate total clinical scores of 5 to 7. Among MVA-BN vaccinated macaques (groups 2, 3, 5, and 6) hunched posturing was also observed in some animals, as well as mild-to-moderate inappetence and mild-to-moderate coughing. Group 5 macaque #A12018 developed a transient dyspnea on Day 70, otherwise dyspnea was absent in vaccinated animals. Other symptoms (nasal discharge, edema, ocular discharge) were either absent or were mild and transient in vaccinated macaques. MVA-BN vaccinated macaques had significantly lower total clinical scores than TBS-treated macaques (Figure 16).

In the high-dose challenge cohort, 5/6 (83%) of macaques treated with TBS (group 4) succumbed to MPXV infection. Group 4 macaque #A11864 had a total clinical score of 8 on Day 74, as well as coughing and respiratory distress (open-mouth breathing) with a total clinical score of 10 on Day 81, and an extended period of dehydration through Day 89, but survived MPXV challenge. Thus, MVA-BN vaccinated macaques were protected from MPXV morbidity and mortality compared to TBS-treated animals.

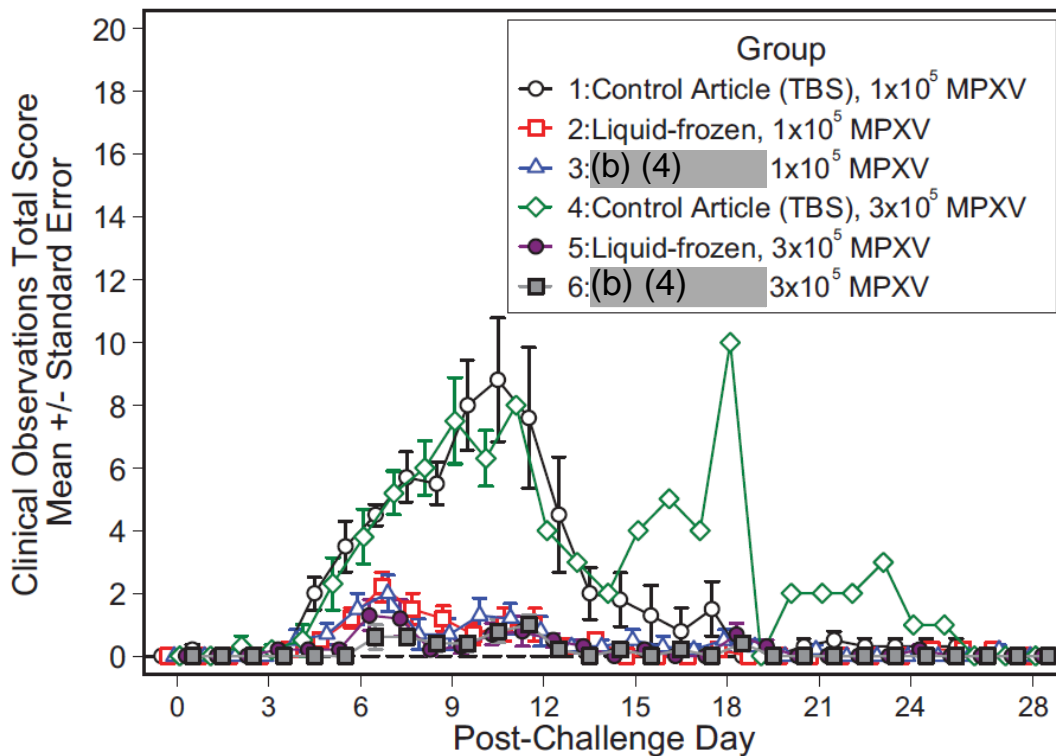


Figure 16. Group Mean Total Clinical Scores (\pm SEM) After MPXV Challenge of Macaques in Study BN-PRE-11-021.

Body Weight and Body Temperature

Generally, peak weight losses were higher in TBS-treated macaques than those in the vaccinated cohort. Peak weight losses of between 2.7 to 14% and 3.4 to 12.9% were recorded among macaques in groups 1 and 4, respectively, but was transient or at baseline in MVA-BN vaccinated animals. The applicant indicated however, that detailed statistical comparisons of weight records were not performed due to irregular weight changes recorded at some timepoints. For example, a weight change of $>10\%$ between two consecutive timepoints for an individual macaque.

Body temperature changes were similar in all treatment groups, with transient increases recorded on Days 69, 81, 84, and 87. A similar pattern was recorded in temperature chips inserted in the left hips (hip temperature) and those inserted in the shoulders (shoulder temperature). However, decrease in body temperature was recorded in macaques that succumbed to infection. Macaque #A11864 (group 4 survivor) also experienced decreases in body temperature from Day 69 to Day 89. Consistent with the observation in other studies above, a decrease in body temperature appears to be predictive of disease severity and the likelihood of mortality in the NHP/MPXV challenge model.

Pock Lesions

No pock lesion was observed in any group through Day 69. By Day 72, five of six macaques in group 1 had at least one lesion (range, 1 to 26 lesions) in one part of the body (arms, legs, head, ventral torso, and dorsal torso). Group 1 macaque # A10080 that was euthanized on

Day 73 had no detectable lesion. Low numbers of lesions (range, 2 to 29) remained detectable in the four group 1 survivors through Day 81 and in two macaques through Day 87, but were resolved by Day 91 (Figure 17; page 568, and Appendix G of Study Report BN-PRE-11-021 in Amendment 28 to STN 125678). By contrast, vaccinated macaques in the low-dose challenge cohort (groups 2 and 3) had no detectable lesions except for group 2 macaque #A11330 that had a total of six lesions (five on the arms and one on a leg) on Day 72, and macaque A12021 in the same group that had two arm lesions on Day 75. These exceptions were free of lesions at other timepoints. In the high-dose challenge cohort, two of the macaques that succumbed in the TBS group had no lesions but the other three had lesions in at least one part of the body (range, 1 to 12) on Day 72. The lone group 4 survivor had lesions at different time points from Day 72 the end of the study on Day 91. In the MVA-BN vaccinated groups 5 and 6 macaques, a macaque in each group had a lesion on a leg on Day 72, otherwise there were no lesions detected in any part of the body at any timepoint.

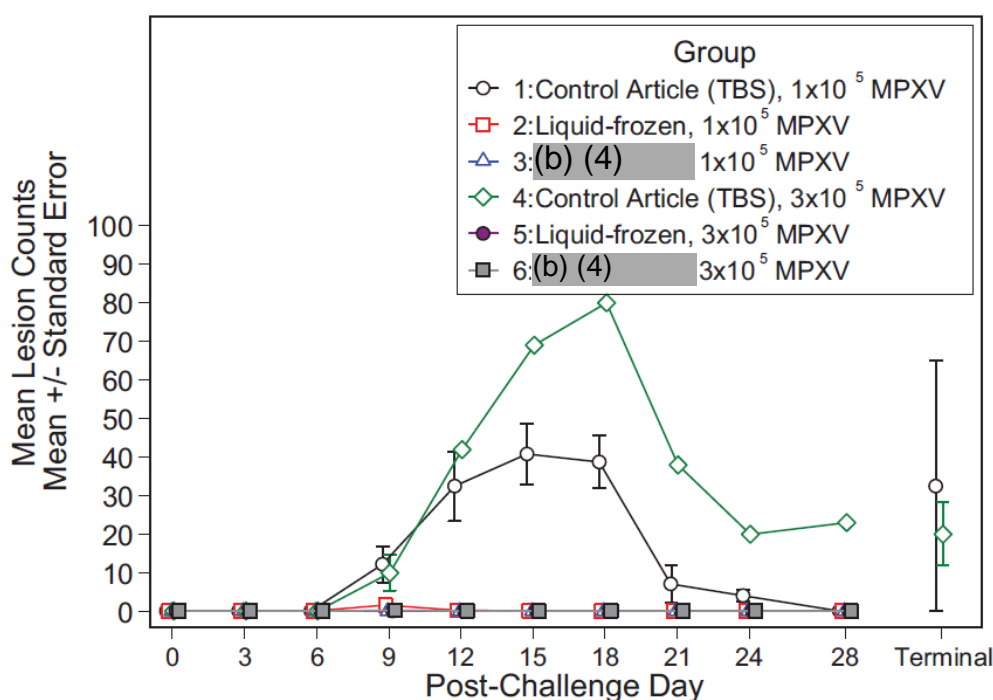


Figure 17. Group Mean Lesion Counts (\pm SEM) After MPXV Challenge of Macaques in Study BN-PRE-11-021. The Mean Terminal Lesion Counts for Macaques That Succumbed in Groups 1 and 4 are Shown as “Terminal”.

Although pock lesion formation does not present as a prominent presentation of MPXV morbidity in the aerosol challenge model as evident in the low number of pock lesions recorded in non-vaccinated animals, the data from this study indicate higher lesion counts and wider body spread of lesions in unvaccinated macaques than the MVA-BN vaccinated cohort.

Blood Viral Load

In the low-dose challenge group, five of six macaques in group 1 had detectable MPXV genome by Day 66 and all had viremia by Day 69. The group viral load peaked on Day 72, with a mean of 1.38×10^7 (range, 2.88×10^6 to 4.57×10^7) genome copies/mL, and the two non-

survivors had blood viral loads of 1.26×10^7 and 2.45×10^7 genome copies/mL (Figure 18, page 617 and Appendix J of Study Report BN-PRE-11-021 in Amendment 28 to STN 125678). In the MVA-BN vaccinated cohort (groups 2 and 3), peak viremia was recorded on Day 69. The mean peak MPXV load for groups 2 and 3 were 6.92×10^2 and 1.23×10^4 genome copies/mL, respectively. Two macaques each in groups 2 and 3 had no detectable viremia or were below the limit of quantitation (LOQ; 8×10^2 genome copies/mL). All other macaques in the MVA-BN vaccinated/low-dose challenge cohort had low levels of transient viremia between Days 69 and 72 (range, 2.5×10^3 to 4.9×10^5 genome copies/mL) and were all cleared or below LOQ levels by Day 75. Similar to the low-dose challenge cohort, all macaques in the TBS-treated/high-dose challenge group (group 4) had viremia on Day 69. A group mean peak of 1.3×10^7 (range, 5.2×10^6 to 4.9×10^7) genome copies/mL was recorded on Day 72. The lone group 4 survivor had a peak load of 1.6×10^7 genome copies/mL on Day 75 and continued to shed virus through Day 91 (8.7×10^2 genome copies/mL). Two macaques in each of groups 5 and 6 (vaccinated) had no viremia or were below LOQ levels at all timepoints.

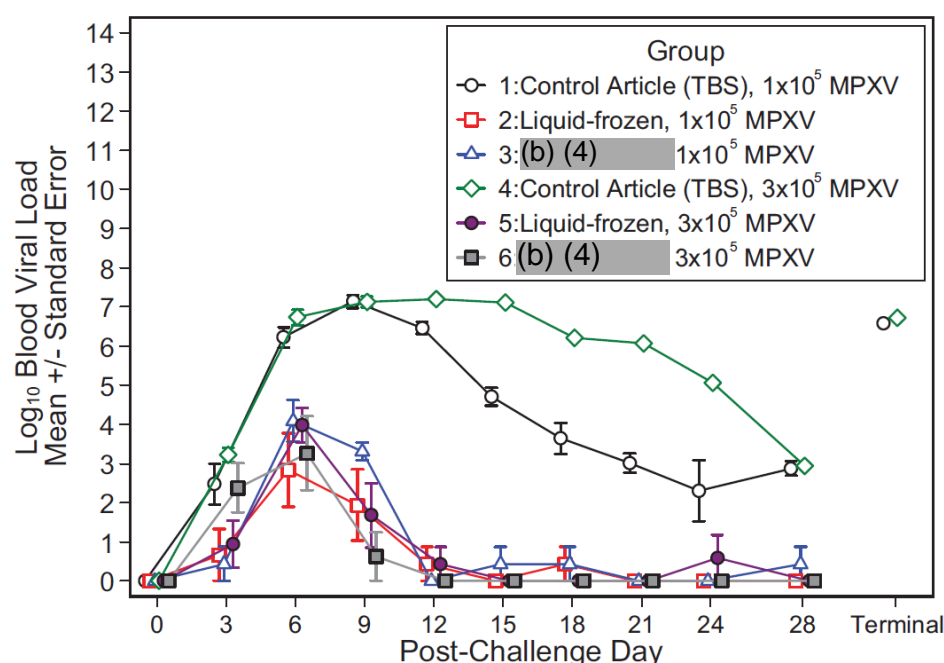


Figure 18. Group Mean Blood Viral Load (\pm SEM) After MPXV Challenge of Macaques in Study BN-PRE-11-021. The Mean Terminal Viral Loads for Macaques That Succumbed in Groups 1 and 4 are Shown as “Terminal”.

The remaining animals in the vaccinated cohort had low levels of transient viremia from Day 66 to Day 72 (range, 1.1×10^3 to 1.3×10^5 genome copies/mL). Apart from group 5 macaque #A12018, which had transient viremia (3.4×10^3 genome copies/mL) on Day 87, all vaccinated macaques had no viremia or below LOQ level from Day 75 to the termination of the study.

Throat Virus Shedding

Throat virus shedding data is presented in Figure 19 (page 626 of Study Report BN-PRE-11-021 in Amendment 28 to STN 125678). One unvaccinated (group 1) macaque in the low-dose challenge group was shedding MPXV on Day 63 and two others were shedding at levels below the LOQ (125 genome copies/mL) at this timepoint. All group 1 macaques had detectable

genome copies by Day 66 (Pages 633 to 634, Appendix J of Study Report BN-PRE-11-021 in Amendment 28 to STN 125678). A group peak shedding of 4.4×10^5 (range, 7.1×10^4 to 2.1×10^6 genome copies/mL) was recorded on Day 72 but a higher mean peak of 1.9×10^6 genome copies/mL was obtained for the remaining five macaques on Day 75. The two macaques that succumbed had 4.9×10^6 and 3.7×10^7 genome copies/mL at the time of euthanasia on Days 73 and 75, respectively. Except for group 1 macaque #A11873 that had no detectable MPXV genome in its throat swab sample on Day 91, survivors remained MPXV-shedding through the end of the study. Three (3/6) macaques and one (1/5) macaque in groups 2 and 3, respectively, were shedding MPXV on Day 63. By Day 66, all macaques in the vaccinated cohort were shedding virus. Group 2 virus shedding peaked at 2.8×10^4 (range, 3.9×10^3 to 4.2×10^4) genome copies/mL on Day 75, and group 3 virus shedding peaked at 3.9×10^4 (range, 9.3×10^3 to 3.2×10^5) genome copies/mL on Day 66.

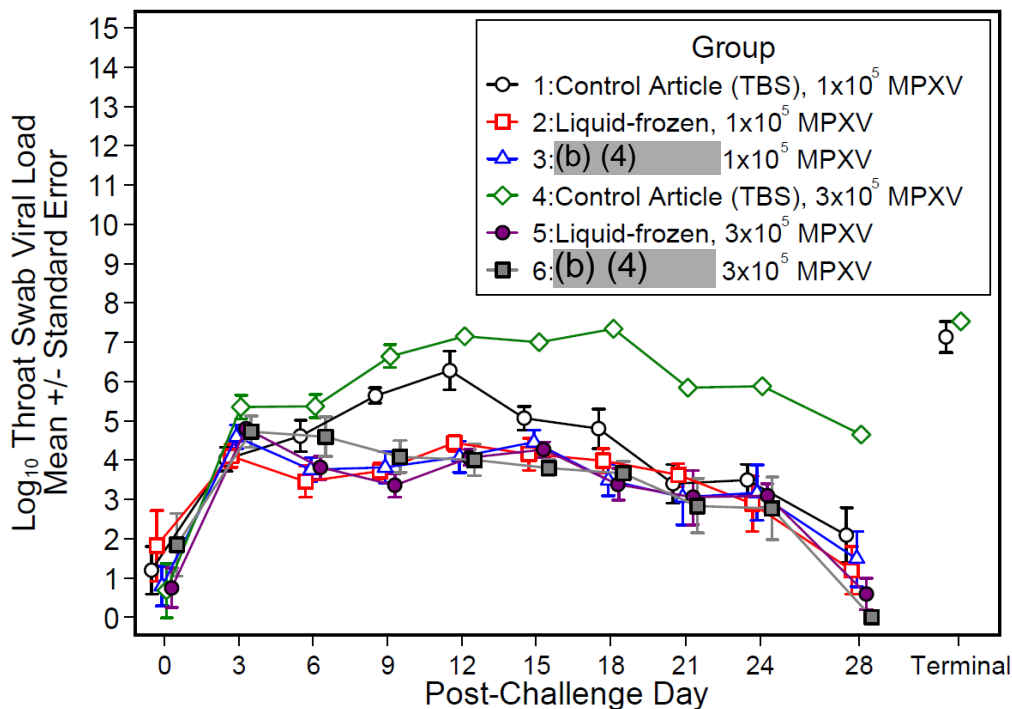


Figure 19. Group Mean Throat Virus Shedding (\pm SEM) After MPXV Challenge of Macaques in Study BN-PRE-11-021. The Mean Terminal Viral Loads for Macaques That Succumbed in Groups 1 and 4 are Shown as “Terminal”.

In the high-dose challenge cohort, a macaque in the TBS-treated group (group 4) had detectable virus shedding on Day 63 and all in the group were shedding virus on Day 66. A group mean peak of 4.5×10^6 (range, 5.0×10^5 to 3.4×10^7) genome copies/mL was recorded on Day 72. The lone survivor had a peak of 3.4×10^7 genome copies/mL on Day 72 and remained virus-shedding through Day 91 (4.5×10^4) genome copies/mL. One macaque in group 5 and two in group 6 had detectable MPXV genome on Day 63 and all vaccinated macaques (groups 5 and 6) were shedding virus from Day 66. Peak virus shedding for group 5 (6.3×10^4 ; range, 3.9×10^4 to 1.4×10^5) genome copies/mL and group 6 (5.4×10^4 ; range, 1.6×10^3 to 4.1×10^5) genome copies/mL were recorded on Day 66. Five (5/6) macaques in group 5 remained virus-shedding through Day 87 but were all resolved or below LOQ by Day 91. Similarly, 3/5 group 6 macaque shed virus through Day 87, but all had resolved MPXV infection by Day 91.

Pathology

Pathological findings upon necropsy of macaques that succumbed (unscheduled euthanasia) revealed numerous gross lesions, including group 1 macaque #A10080 and group 4 macaque #s A09856 and A10750 that succumbed without developing detectable skin pock lesions. In the TBS/low-dose challenge group (group 1), resolving pocks in the large intestine and skin, granular spleen and enlarged cervical lymph node was found in one of two macaques that succumbed. Discoloration of the lungs, enlarged bronchial lymph node and pocks in the tongue were found in both non-surviving group 1 macaques. Gross lesions found in the five unvaccinated macaques that succumbed in group 4 included: brain foci (2 macaques), fluid in the pericardial cavity (3 macaques), heart foci (1 macaque), pocks in the large intestine (3 macaques), lungs discoloration (all 5 macaques), pocks in the lungs (1 macaque), enlarged bronchial lymph node (4 macaques), skin pocks (3 macaques), spleen foci (1 macaque), and pock in the tongue (1 macaque). Gross lesions detected in macaques that survived MPXV challenge, including the four group 1 and single group 6 survivors, were fewer and mostly observed in one macaque in each treatment group, and include resolving pocks on skin, lungs and spleen, and lymphadenitis.

Review Comment: Although the main objective of this study was to compare the immunogenicity and protective efficacy of the LF (b) (4) formulations of MVA-BN in the aerosolized MPXV challenge model, the data obtained further underscored the immunogenicity and protective efficacy of MVA-BN in this preclinical model, irrespective of the vaccine formulation.

Study BN-PRE-12-028 (NHP/MPXV Aerosol Challenge)

Title

Comparison of the Efficacy and Immunogenicity of Liquid-frozen (b) (4) Formulations of IMVAMUNE® in the Aerosol Monkeypox Challenge Model in Cynomolgus Macaques

Study Design Summary

Study BN-PRE-12-028 was conducted under study number 2812-100011360 at BBRC. The study was similar in design, objectives and execution to study BN-PRE-11-021, except that Study BN-PRE-12-028 contained three treatment groups, each with increased number of macaques, and a single target challenge dose of 3×10^5 PFU of aerosolized MPXV was used (Table 21; adapted from page 16 of Study Report BN-PRE-12-028 in STN 125678/0).

Test articles, including clinical-grade TBS (Lot # (b) (4)), MVA-BN-LF (Lot # C00004) and MVA-BN^{(b) (4)} (Lot # (b) (4)) were supplied by the applicant. MPXV ((b) (4)) was obtained from (b) (4). Pre- and post-vaccination blood samples collected from animals were tested for antibodies by ELISA and PRNT.

Thirty-six (18 males/18 females) healthy, specific pathogen-free macaques weighing 2.4-3.5 kg and aged 2.25 to 5 years were sourced from (b) (4). Macaques were quarantined and acclimatized at BBRC. A female macaque (# A13376) was found to have

alternating arm seizures of unknown cause and was excluded from the study. Thus, thirty-five (35) macaques were randomized by weight and sex into three treatment groups and vaccinated with MVA-BN formulations or treated with TBS (Table 21).

Table 21. Design of Study BN-PRE-12-028

Group	No. of macaques	Vaccination (Day 0 and Day 28)	Vaccine Dose (TCID ₅₀)	Route	Vaccination schedule (study day)	MPXV challenge on day 63 (PFU)
1	10	TBS	N/A	SC	0, 28	3x10 ⁵
2	12	MVA-BN LF	1x10 ⁸	SC	0, 28	3x10 ⁵
3	13	MVA-BN (b) (4)	1x10 ⁸	SC	0, 28	3x10 ⁵

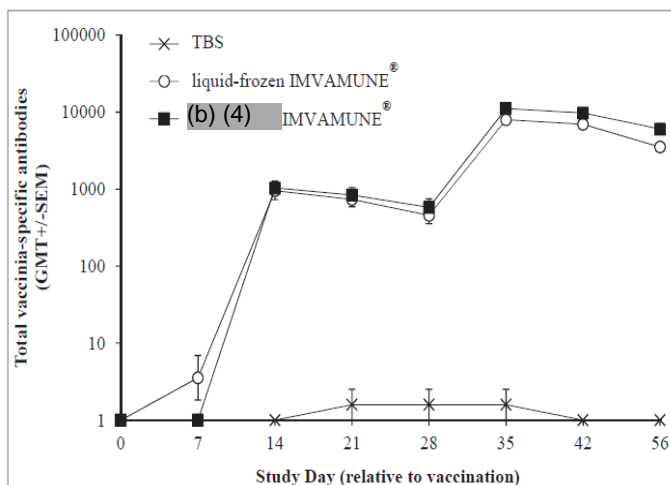
N/A, Not Applicable; TCID₅₀, 50% Tissue Culture Infectious Dose; SC, Subcutaneous.

Pre- and post-vaccination serum samples were tested for binding and neutralizing antibodies, and each macaque was exposed to a target dose of 3x10⁵ PFU of MPXV by a single-animal head-only exposure to aerosolized MPXV for inhalational infection. Aerosolized MPXV was generated as described under study BN-PRE-11-021 above. Blood samples and throat swabs for the assessment of viremia and virus shedding were collected on Day 63, and every 3 days through day 87 and day 91 (subgroup CDA) or day 92 (subgroup CDB). After MPXV challenge, macaques were observed for clinical signs, MPXV morbidity and mortality as described under study BN-PRE-11-021.

Immunogenicity Results

Macaques in all treatment groups had no detectable vaccinia-specific antibodies in pre-vaccination (Day 0) serum samples. Three group 2 macaques had seroconverted with low (≤ 200) IgG titers on Day 7, and by Day 14, all macaques vaccinated with MVA-BN (groups 2 and 3) had seroconverted with mean ELISA GMTs of 953 (range, 200 to 3242) and 1033 (range,

ELISA GMTs



PRNT GMTs

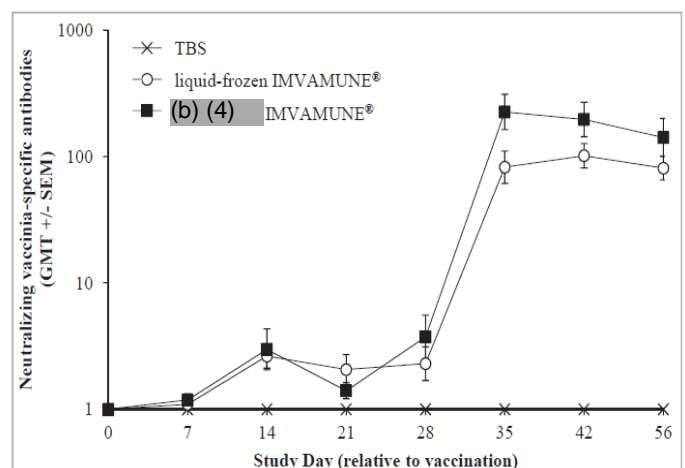


Figure 20. Immunogenicity of MVA-BN LF and MVA-BN (b) (4) in Study BN-PRE-12-028

418 to 5376) for the MVA-BN LF and MVA-BN (b) (4) formulations, respectively (Figure 20; pages 545, and Appendix 1, pages 550 to 556 of Study Report BN-PRE-12-028 in STN 125678/0). Macaque #A11224 in the TBS group (group 1) had an IgG titer of 100 on Days 21 and 28. After booster vaccinations on Day 28, macaque #A11224 remained seropositive on Day 35 (titer = 100) but was seronegative on Days 42 and 56. ELISA antibody titers peaked in both MVA-BN groups on Day 35 with GMTs of 7951 (range, 4351 to 16521) and 11096 (range, 4134 to 23848) for groups 2 and 3, respectively.

Neutralizing antibodies were not detected at any timepoint in any group 1 macaque and all pre-vaccination serum samples. On Day 7, group 2 macaque #A11692 and group 3 macaque #s A12277 and A13373, all had a PRNT titer of 3. However, none of these three macaques had detectable IgG on Day 7, and three group 2 macaques that were seropositive by ELISA on Day 7 had no neutralizing antibodies at this timepoint. Low titers of neutralizing antibodies (a GMT range of 2 to 4) were detected in vaccinated macaques (groups 2 and 3) at various timepoints from Day 7 to 28, with PRNT seroconversion rate of between 8 to 75% (Figure 20; page 546, and Appendix 2, pages 559 to 567 of Study Report BN-PRE-12-028 in STN 125678/0). In group 2, peak PRNT neutralizing antibodies was attained on Day 42 with a GMT of 101 (range, 22 to 271), while in group 3, macaques attained peak PRNT titer on Day 35, with a GMT of 226 (range, 36 to 988) (Figure 20).

Results of MPXV Challenge (MPXV Morbidity and Survival)

The mean actual delivered dose of aerosolized MPXV over the two challenge days (CDA and CDB) was 5.88×10^5 PFU (range, 2.03×10^5 to 1.38×10^6 PFU) (page 25 and Appendix P (page 589) of Study Report BN-PRE-12-028 in STN 125678/0). Following aerosol challenge with

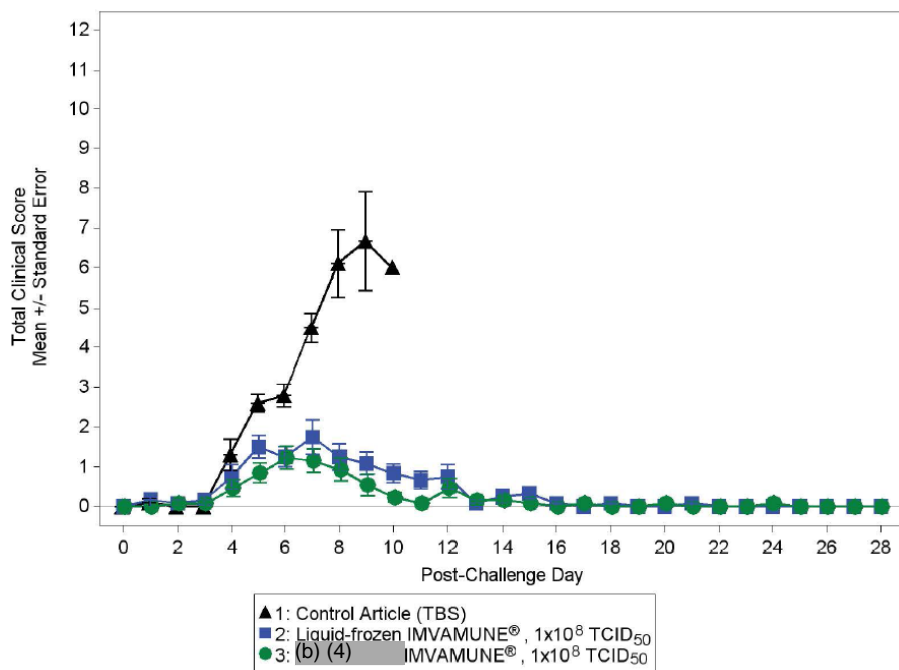


Figure 21. Mean Total Clinical Scores (± SEM) After MPXV Challenge in Study BN-PRE-12-028.

MPXV, mild clinical symptoms (inappetence, hunched posture) appeared in some macaques in the TBS group four days later (Day 67), and all 10 macaques had developed symptoms by

Day 68. Moderate-to-severe hunched posture, moderate inappetence, mild-to-moderate dehydration (macaque #A12395 experienced severe dehydration), and respiratory distress (coughing (7/10) and dyspnea (5/10)). In addition, nasal discharge and ocular discharge occurred in 4/10 and 3/10 macaques, respectively, with two macaques having both. Clinical signs (hunched posture, inappetence and dehydration) were also recorded in vaccinated macaques from Day 67 to 73 but were milder than group 1. Short periods of infrequent cough were observed in 6/12 group 2 macaques and in 4/13 group 3 macaques. Nasal or ocular discharges were not recorded in any vaccinated macaque. Four group 1 macaques also had mild edema. Clinical signs were mild in MVA-BN vaccinated groups 2 and 3 macaques, with a group 2 macaque developing mild edema. Mean total clinical scores after MPXV challenge indicated a peak mean score of 6.7 (range 5 to 11) on Day 72 in TBS-treated macaques (Figure 21, page 386 of Study Report BN-PRE-12-028 in STN 125678/0).

Among vaccinated macaques, total clinical scores ranged from 1 to 4 (group 2) and 1 to 3 (group 4). All ten group 1 macaques succumbed to MPXV infection (four were euthanized on Days 71 and 72, and six were found dead between Days 71 to 74) (Table 22, adapted from: pages 171; pages 391 to 394 (Appendix G); and pages 429 to 432 (Appendix J) of Study BN-PRE-12-028 in STN 125678/0). All macaques vaccinated with MVA-BN, irrespective of the formulation, survived.

Table 22. MPXV Morbidity and Mortality in Study BN-PRE-12-028

Group	No. of macaques	Vaccination (Schedule Day)	Aerosol MPXV Challenge on Day 63 (PFU)	Peak Mean Total Body Lesion Count	Macaques without pock lesions	Peak Mean Blood MPXV Load (genome copies/mL)	No of macaques without viremia at any timepoint	Peak Mean Throat MPXV Shedding (genome copies/mL)	Survival (%)
1	10	TBS	3x10 ⁵	47.1	3 ^a	1.1x10 ⁷	0/6	4.4x10 ⁵	0/10 (0)
2	12	MVA-BN LF	3x10 ⁵	0.3	11/12 ^b	6.9x10 ²	2/6	2.8x10 ⁴	12/12 (100)
3	13	MVA-BN ^{(b) (4)}	3x10 ⁵	0.1	12/13 ^c	1.2x10 ⁴	0/6	3.9x10 ⁴	13/13 (100)

^aPock lesions did not develop in three macaques that succumbed (2 found dead, 1 euthanized) on Day 81

^bA macaque had a total of 3 lesions on Day 75 and 1 lesion on Day 78

^cA macaque had one lesion on Day 81

Body Weight and Body Temperature

Weight loss was recorded among unvaccinated group 1 macaques with a peak mean weight loss of 5.4% (range, 2.1% to 8.8%) on Day 72. Three of twelve (3/12) group 2 macaques did not lose weight but 9 macaques lost between 1% to 5.7% at different timepoints. Group 2 macaque # A12294 experienced weight loss throughout the study and was 6% below baseline weight at the termination of study on Day 91. Nine of the thirteen (9/13) macaques in group 3 did not lose weight but the remaining four lost 1.9% to 3.2% of their weight at different timepoints between Day 69 to 81.

Consistent with the temperature decline in unvaccinated macaques as observed in study BN-PRE-11-021, TBS-treated macaques experienced a decline in body temperature after MPXV

challenge, while vaccinated macaques in groups 2 and 3 experienced minor body temperature changes from baseline, both in the shoulder and hip measurements.

Pock Lesions

MPXV pock lesions were detected in three group 1 macaques on Day 69 and peaked on Day 72 with a mean peak count of 47.1 lesions (Table 22). Three group 1 macaques had no detectable pock lesions at any timepoint before they succumbed on Day 81. Peak mean lesion counts for groups 2 and 3 were 0.3 (Day 75) and 0.1 (Days 75, 78 and 81), respectively. In the applicant's analysis, there was a significant difference in pock counts between unvaccinated (group 1) and vaccinated (groups 2 and 3) macaques ($p=0.0000002514$).

Blood Viral Load

By Day 66, eight of ten (8/10) unvaccinated (group 1) macaques had viremia and by Day 69, all were viremic. A peak blood virus load of 1.1×10^7 (range, 1×10^6 to 4.6×10^8) genome copies/mL (representing the mean for six macaques) was recorded on Day 72 (Table 22; and Figure 22 (page 433) and Appendix J (pages 429 to 430) of Study Report BN-PRE-12-028 in STN 125678/0). Two group 2 macaques had no detectable MPXV genome at any timepoint, and two others had levels below the LOQ (8×10^2 copies/mL) on Days 66 and 69 but otherwise had no detectable MPXV genome. Peak viremia occurred on Day 69 with a group mean genome copy of 3.8×10^3 (range, below LOQ 1.1×10^6) genome copies/mL. Most group 2 macaques had no detectable MPXV genome from Day 75 except for a couple with transient

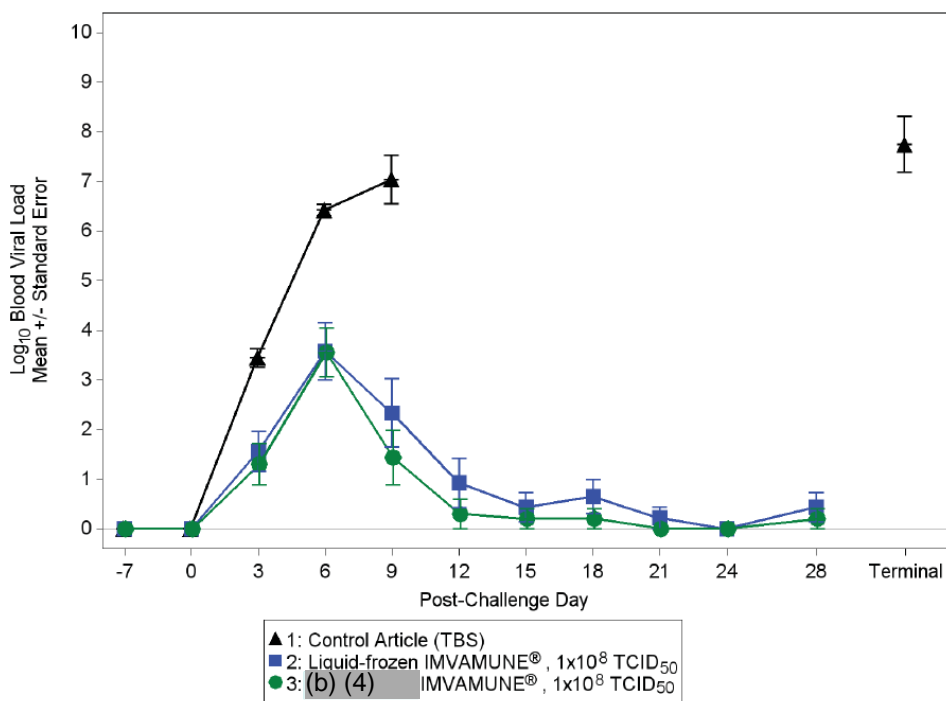


Figure 22. Mean Group Blood Viral Load (\pm SEM) After MPXV Challenge in Study BN-PRE-12-028. The Mean Terminal Viral Load for Macaques That Succumbed in Group 1 is Shown as “Terminal”.

below-LOQ levels at different timepoints. In group 3, two macaques had no viremia at any timepoint and peak viremia was also recorded on Day 69 with a group mean of 3.6×10^3 (range, below LOQ 1×10^6) genome copies/mL. With the exception of macaque #A11217, all macaques had no detectable MPXV genome from Day 75. Macaque A11217 had below LOQ levels on Days 78, 81 and 91. The applicant's analysis indicate statistically significant difference in viremia between vaccinated (groups 2 and 3) and unvaccinated (group 1) macaques on Days 66, 69, and 72.

Throat Virus Shedding

Most macaques in the study (9/10 TBS group; 10/12 MVA-BN LF; and 10/13 MVA-BN ^{(b) (4)}) had detectable throat virus shedding on Day 66. By Day 69, macaques in all treatment groups were shedding virus, albeit a macaque in each of groups 2 and 3 had below LOQ levels of MPXV genome. A peak virus shedding of 6.9×10^6 (range, 5.6×10^5 to 8.3×10^7) genome copies/mL (representing the mean for six macaques) was recorded on Day 72 (Table 22; and Figure 23, page 437, Appendix J (pages 431 to 432) of Study Report BN-PRE-12-028 in STN 125678/0). Peak virus shedding in group 2 was recorded on Day 69, with a group mean of 7.4×10^3 (range, 8.3×10^1 to 2.5×10^5) genome copies/mL. On Day 81 group 2 macaque # A13300 was free of MPXV but other macaques in the group had low levels of transient virus shedding (range, < LOQ to 2×10^3 copies/mL) at different timepoints till the termination of study. In group 3, virus shedding peaked on Day 72 with a mean of 8.5×10^3 (range, 8.3×10^1 to 3.2×10^5) genome

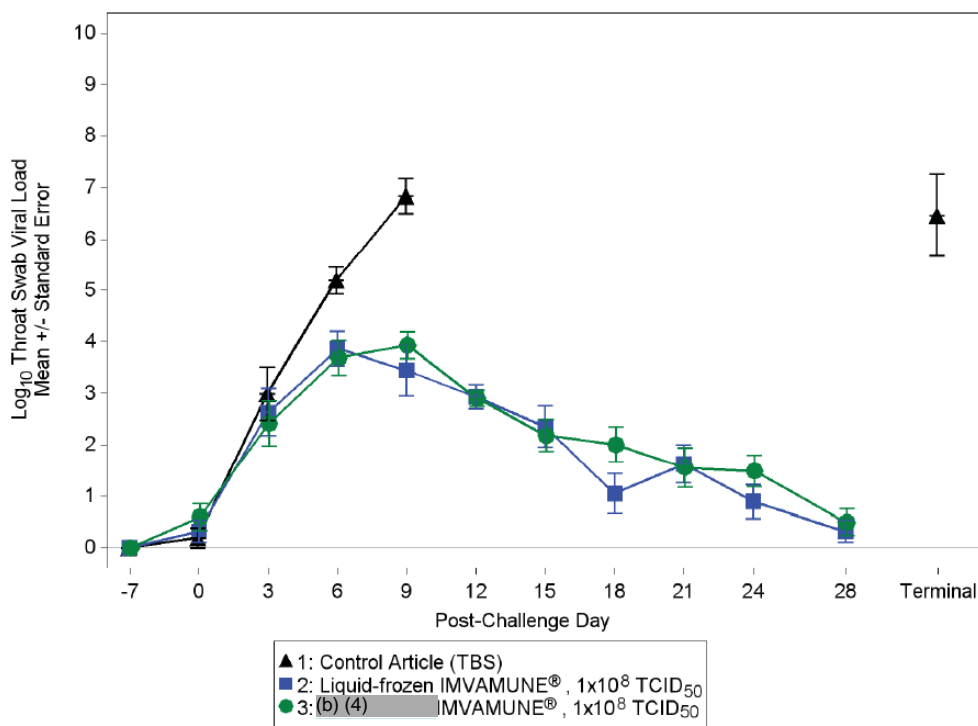


Figure 23. Mean Group Throat Swab Viral Load (± SEM) After MPXV Challenge in Study BN-PRE-12-028. The Mean Terminal Viral Load for Macaques That Succumbed in Group 1 is Shown as “Terminal”.

copies/mL. All group 3 macaques had low levels of transient MPXV genome in throat swab samples (a range of < LOQ to 6.4×10^3 copies/mL) from Day 81 through the end of the study. In

the applicant's analysis, there was a statistically significant difference (p values < 0.05) in throat virus load between vaccinated and unvaccinated macaques on Day 72.

Review Comment: The immunogenicity data obtained in study BN-PRE-12-028 confirmed the ability of MVA-BN to induce antibody responses upon inoculation into macaques, consistent with the outcomes of other study reports above. Data from this study also lend credence to the efficacy of MVA-BN in protecting macaques from morbidity and mortality caused by MPXV in the aerosol challenge model..