BLA Clinical Review Memorandum

Application Type	Biologics License Application (BLA)
STN	125678/0
CBER Received Date	10/25/2018
PDUFA Goal Date	9/24/2019
Division / Office	Division of Vaccines and Related Product Applications (DVRPA) Office of Vaccines Research and Review (OVRR)
Priority Review (Yes/No)	Yes
Reviewer Name(s)	Sixun Yang, MD, PhD Alexandra Yonts, MD
Review Completion Date / Stamped Date	7/15/2019/ 9/19/2019
Supervisory Concurrence	Andrea Hulse, MD, Branch Chief
Applicant	Bavarian Nordic A/S
Established Name	Smallpox and Monkeypox Vaccine, Live, Non-replicating
Approved Trade Name	JYNNEOS
Pharmacologic Class	Prophylactic Vaccine
Formulation(s), including Adjuvants, etc.	Sterile, Liquid-Frozen Suspension Containing A Dose of 0.5 mL with At Least 0.5×10^8 Infectious Units of Modified Vaccinia Ankara-BN (MVA-BN)
Dosage Form(s) and Route(s) of Administration	0.5 mL, Subcutaneous Injection
Dosing Regimen	Two doses, 28 days apart
Indication(s) and Intended Population(s)	Active immunization against smallpox and monkeypox in individuals 18 years of age and older
Orphan Designated (Yes/No)	No

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GLOSSARY

AAR	area attenuation ratio
ACIP	Advisory Committee on Immunization Practices
ACV	assay cut-off value
AD	atopic dermatitis
AE	adverse event
AESI	adverse evet of special interest
AIDS	acquired immune deficiency syndrome
BLA	biologics license application
BN	Bavarian Nordic
BPCA	Best Pharmaceuticals for Children Act
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
cGMP	current good manufacturing practice
CI	confidence interval
CMC	chemistry, manufacturing, and controls
CSR	clinical study report
DIS	Division of Inspections and Surveillance
ECTV	ectromelia virus
eCTD	electronic Common Technical Document
ELISA	Enzyme-Linked Immunosorbent Assay
ELISPOT	Enzyme-Linked Immuno Spot Assay
ES	Executive Summary
EUA	emergency use authorization
FAS	full analysis set
(b) (4)	
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
GMT	geometric mean titer
GRMP	good review management principles
HIV	human immunodeficiency virus
ICH	International Conference on Harmonization (of Technical Requirements for Registration of Pharmaceuticals for Human Use)
IM	intramuscular
Inf.U	Infectious Units
ISE	integrated summary of efficacy
ISS	integrated summary of safety
ITRC	Independent Take Review Committee
ITT	intent-to-treat
LB	lower bound
LF	liquid-frozen LLOD lower limit of detection
LLOQ	lower limit of quantitation
MedDRA	Medical Dictionary for Regulatory Activities
Max	maximum
MI	myocardial infarction

Min	minimum
MLA	maximum lesion area
MLD	maximum lesion diameter
MPXV	monkeypox virus
MVA	Modified Vaccinia Virus Ankara strain
MHP	non-human primate
NIH	National Institutes of Health
OBE	Office of Biostatistics and Epidemiology
OCOD	Office of Communication Outreach and Development (CBER)
OSE	Office of Surveillance and Epidemiology
PD	pharmacodynamics
PeRC	Pediatric Review Committee (CDER)
pfu	plaque forming units
PI	package insert
PK	pharmacokinetics
PMC	postmarketing commitment
PMR	postmarketing requirement
PPS	per protocol set
PREA	Pediatric Research Equity Act
PRNT	plaque reduction neutralization test
REMS	risk evaluation and mitigation strategy
RMS/BLA	regulatory management system for the biologics license application
RTF	refuse to file
SAE	serious adverse event
SC	subcutaneous
SCR	seroconversion rate
SD	standard deviation
SFU	spot forming unit
SOP	standard operating procedure
$TCID_{50}$	tissue culture infectious dose$_{50}$
ULN	upper limit of normal
US/USA	United States of America
VV	Vaccinia Virus
VV-WR	Vaccinia Virus, Western Reserve strain
WHO	World Health Organization

1. EXECUTIVE SUMMARY

JYNNEOS, also referred to as Modified Vaccinia Virus Ankara–Bavarian Nordic (MVA-BN), is a highly-attenuated vaccinia virus derived from strain MVA-572 and does not replicate in human cells. MVA-BN is indicated for protection against smallpox and monkeypox in individuals 18 years of age and older.

Smallpox is a highly contagious infectious disease caused by variola virus with a mortality rate of 30-40%. Smallpox was declared officially eradicated in 1980. Following the official declaration of smallpox eradication, routine vaccination

programs against smallpox were discontinued, leading to a growing majority of the world's population lacking immunity to smallpox. The intentional release of variola virus, a recognized agent of potential bioterrorist intent, could therefore have devastating effects. The only currently licensed smallpox vaccine, ACAM2000, is a live, replicating vaccinia virus based smallpox vaccine. ACAM2000 is contraindicated in severely immunocompromised individuals who are not expected to benefit from the vaccine. ACAM2000 is also limited to use in individuals at high risk of smallpox because of severe side effects, such as progressive vaccinia in less severely immunocompromised individuals for whom the vaccine is not contraindicated, eczema vaccinatum in individuals with atopic dermatitis, myopericarditis in smallpox vaccine naïve individuals, fetal vaccinia in pregnant women, and spread of vaccine virus beyond the vaccination site (generalized vaccinia) or to contacts of vaccinees. Therefore, an unmet medical need exists for a smallpox vaccine with an improved safety profile.

Monkeypox is a rare viral zoonosis with symptoms similar to those seen in smallpox patients. Although it is clinically less severe than smallpox, it can be fatal. Case fatality in monkeypox outbreaks has been between 1% and 10%. With the eradication of smallpox in 1980 and subsequent cessation of smallpox vaccination, monkeypox virus has emerged as the most important orthopoxvirus. Monkeypox occurs sporadically in central and western Africa's tropical rainforest. A monkeypox outbreak was first confirmed in the U.S. in 2003. There is no specific treatment or approved vaccine for monkeypox although Advisory Committee on Immunization Practices (ACIP) recommends that ACAM2000 be used for prevention of monkeypox in individuals at high risk of exposure (e.g., lab workers who handle monkeypox virus).

BN (also referred to as the applicant throughout the document) proposed a 2-dose primary series for use in smallpox vaccine naïve individuals and a single booster dose for use in individuals previously vaccinated with a smallpox vaccine (replicating smallpox vaccine or MVA-BN primary series). They submitted 22 clinical trials to support the effectiveness and safety of MVA-BN for licensure. Among these 22 clinical trials, 7 clinical trials are considered essential by the review team to support the proposed indication and usage. Full clinical study reports were submitted to the BLA for these 7 studies:

- POX-MVA-006: A pivotal Phase 3 non-inferiority trial comparing MVA-BN with ACAM2000 to support safety and effectiveness of MVA-BN in vaccinia naïve healthy subjects
- POX-MVA-013: A placebo-controlled Phase 3 lot consistency trial to establish manufacturing consistency of MVA-BN as well as to support safety of MVA-BN
- POX-MVA-008: A Phase 2 trial to support use of MVA-BN in individuals with atopic dermatitis
- POX-MVA-011: A Phase 2 trial to support use of MVA-BN in HIV-infected individuals

- POX-MVA-005 and -23: A Phase 2 trial and its extension trial, respectively, to support use of MVA-BN in vaccinia experienced individuals
- POX-MVA-024: A Phase 2 trial to support use of MVA-BN in individuals 65 years of age and older

In addition to these essential clinical trials, BN submitted an integrated summary of safety, pooled SAEs and cardiac AESIs across various study populations throughout the clinical development program.

During the discussions of licensure pathway for MVA-BN, we agreed that the most appropriate approach to licensure for MVA-BN would be to demonstrate vaccine effectiveness compared to ACAM2000 using a primary endpoint of non-inferior vaccinia specific neutralizing antibody titers. The non-inferiority margin was pre-specified at 0.5. Given that vaccine antigens and replication competence are different for MVA-BN vs. ACAM2000, and that a vaccinia neutralizing antibody response that predicts protection against smallpox has not been established, we considered that demonstrating vaccine efficacy in animal models showing protection against relevant orthopoxvirus challenge (e.g., monkeypox in NHPs) would be critical to support the immunologic non-inferiority comparison.

The applicant had also proposed ACAM2000 take attenuation following MVA-BN vaccination as a co-primary endpoint that provided clinically meaningful evidence that vaccinia virus replication at the ACAM2000 inoculation site was suppressed by the immune response to MVA-BN. While we considered immunologic non-inferiority to ACAM2000, in combination with supportive animal efficacy data, to be adequate to demonstrate vaccine effectiveness, we agreed with the applicant's proposal to include a co-primary endpoint evaluating attenuation of ACAM2000 take reaction in individuals previously vaccinated with MVA-BN compared to smallpox vaccine naïve individuals.

The applicant's original proposed indication did not include monkeypox. During the review of this submission, we received inquiries from external stakeholders in the US government asking whether the available data for MVA-BN would support an indication for prevention of monkeypox. We determined that immunogenicity data for MVA-BN obtained in humans together with the non-human primate (NHP) data already submitted to BLA 125678/0 support the indication for prevention of monkeypox, since the clinical and non-clinical studies provided multiple lines of evidence that the immune response to MVA-BN provided protection against different orthopoxviruses, and specifically monkeypox in the NHP challenge model. Therefore, we recommended including the monkeypox indication in the product labeling.

Summary of Vaccine Effectiveness

Study POX-MVA-006

POX-MVA-006 was a two-site, open-label, randomized, immune-analysis blinded Phase 3 trial to assess the effectiveness and safety of MVA-BN compared to ACAM2000 in approximately 440 smallpox vaccine-naïve, healthy US military personnel 18 through 42 years of age. The co-primary endpoints were vaccinia specific neutralizing antibody titer at Peak Visit, and take attenuation following ACAM2000 scarification in subjects previously vaccinated with MVA-BN. Solicited adverse reactions were collected via diary card for 14 days after each vaccination, and serious adverse events (SAEs) and adverse events of special interest (AESIs) were followed up for at least 6 months after the last vaccination.

The trial included two groups:

- Group 1: Vaccinia-naïve subjects received two 0.5 mL (1×10^8 Inf.U) doses of MVA-BN, administered subcutaneously (SC) four weeks apart followed by one dose of ACAM2000 ($2.5\text{-}12.5 \times 10^5$ plaque forming units) via scarification four weeks after the second MVA-BN vaccination
- Group 2: Vaccinia-naïve subjects received one dose of ACAM2000 ($2.5\text{-}12.5 \times 10^5$ plaque forming units) via scarification

Vaccinia specific neutralizing antibody was determined by plaque reduction neutralization test (PRNT) using the Western Reserve strain of vaccinia virus (VV-WR) as the reporter. Take attenuation was determined by comparing maximal median skin lesion area (MLA) following ACAM2000 scarification in MVA-BN vaccinated subjects in Group 1 with the MLA following ACAM2000 scarification in Group 2 subjects.

The study enrolled 433 vaccinia-naïve subjects from Department of Defense (DoD) personnel, 220 in Group 1 and 213 in Group 2. Overall, the mean subject age was 23.5 years (range: 18-42 years), with most subjects in the 18-24 year age range (69.5% in each group). A greater proportion of subjects was male [365 subjects (84.3%)], White/Caucasian [262 subjects (60.5%)], and Non-Hispanic or Latino [339 subjects (78.3%)]. Age, ethnicity, race and gender were similar between the two groups.

The co-primary endpoints of this study were to demonstrate the efficacy of MVA-BN by assessing non-inferiority of MVA-BN compared to ACAM2000 in terms of vaccinia-specific PRNT geometric mean titer (GMT) at the Peak Visits, defined as two weeks after the second dose of MVA-BN in Group 1, and four weeks after a single dose of ACMA2000 in Group 2, and by showing that vaccination with MVA-BN prior to scarification with ACAM2000 resulted in take attenuation.

PRNT GMTs at Peak Visits for Group 1 and Group 2 were 152.8 (95%CI: 133.3, 175.0) and 84.4 (95%CI: 73.4, 97.0), respectively. The PRNT GMT ratio of Group 1/Group 2 was 1.8 (97.5% CI: 1.49, 2.20). The study met the protocol specified non-inferiority margin of lower bound (LB) of one-sided 97.5% CI > 0.5.

Therefore, it is reasonable to expect that two doses of MVA-BN administered at 28 days apart is as effective as ACAM2000 in prevention of smallpox disease among smallpox vaccine naïve individuals.

The MLA in Group 1 was 0.0 mm^2 (95%CI: 0.0, 1.0), and the MLA in Group 2 was 37.0 mm^2 (95% CI: 33.0, 42.0). The area attenuation ratio (AAR) was defined as 1-(MLA in Group 1/MLA in Group 2). The AAR in MVA-BN immunized subjects was 97.9% with an LB of 95% CI of 96.6%, which met the protocol specified success criterion of LB of 95% CI > 40%.

The clinical reviewer identified several issues with take assessment. Take distribution among Group 1 subjects clustered in terms of study subject identification number as well as ACAM2000 administration date. There was an imbalance in take rate among Group 1 subjects between the two study sites (57% vs. 36%). In addition, among Group 1 subjects who had no take following ACAM2000 vaccination, vaccinia specific antibody titers were lower after ACAM2000 vaccination than prior to ACAM2000, suggesting vaccination failure of unknown cause. The applicant was not able to provide a reasonable or acceptable explanation for these issues. Therefore, we decided the data were not reliable, and since not necessary to demonstrate vaccine effectiveness would not be considered to support licensure or be included in the package insert.

The vaccinia-specific immunogenicity data from POX-MVA-006, together with efficacy data from animal studies showing that MVA-BN confers protection against aerosol or intratracheal monkeypox virus challenge in non-human primates and protection against intranasal ectromelia virus challenge in mice, established that MVA-BN elicits protective immunity against different orthopoxviruses similar to previously licensed smallpox vaccines. On this basis, we inferred effectiveness of MVA-BN for protection from both smallpox and monkeypox in humans.

In addition to POX-MVA-006, the applicant submitted data from several studies intended to support effectiveness claims in specific subpopulations and to support effectiveness of a single booster dose in smallpox vaccine experienced individuals.

<u>Studies POX-MVA-008, -011, -005/-023 and -024</u>

POX-MVA-008 was a Phase 2, multicenter, open-label, healthy control, prospective cohort study to evaluate the safety and immunogenicity of MVA-BN smallpox vaccine in vaccinia-naïve 18-40-year-old subjects with atopic dermatitis (AD). The study enrolled vaccinia-naïve subjects into two groups: healthy subject control (n=282) and subjects with a history of or currently active AD (n=350). Both groups of subjects received two doses of MVA-BN at 28 days apart. The primary endpoint was seroconversion rate (SCR) determined by ELISA at Peak Visit.

POX-MVA-011 was a Phase 2, multicenter, open-label, healthy-control, prospective cohort study to evaluate the safety and immunogenicity of MVA-BN smallpox vaccine in vaccinia-naïve as well as vaccinia experienced HIV infected subjects. All subjects received two doses of MVA-BN at 28 days apart. The study enrolled 581 subjects: 88 vaccinia naïve and 9 vaccinia experienced healthy subjects, 352 vaccinia-naïve and 132 vaccinia experienced HIV infected subjects. The primary objective of this study was to assess the safety of MVA-BN in HIV-infected subjects compared to healthy subjects. Secondary endpoints included ELISA SCR and GMT, and PRNT SCR and GMT.

POX-MVA-005 was a Phase 2 trial to compare immunogenicity of two doses of MVA-BN in vaccinia-naïve healthy subjects and a single dose of MVA-BN in vaccinia-experienced healthy subjects who were vaccinated with the first generation of smallpox vaccines over 25 years ago. The primary endpoint was vaccinia-specific SCR derived from the ELISA specific antibody titers two weeks after the last vaccination. The study enrolled 549 vaccinia-naïve subjects and 204 subjects who were previously vaccinated with the first generation of smallpox vaccines.

POX-MVA-023 was an extension study of POX-MVA-005 to evaluate the safety and immunogenicity of a single dose of MVA-BN in MVA-BN experienced subjects. POX-MVA-023 also evaluated persistence of immune responses following the primary MVA-BN vaccination as well as following a single booster dose vaccination with MVA-BN among MVA-BN primed subjects and subjects who received replicating vaccinia based smallpox vaccines. The primary endpoint was vaccinia-specific SCR derived from the ELISA specific antibody titers two weeks after the last vaccination.

POX-MVA-024 was a randomized, double-blind, placebo-controlled study to evaluate the safety and immunogenicity of one versus two doses of MVA-BN among 120 subjects 56 - 80 year- old who were previously vaccinated with smallpox vaccines. The primary objective was safety. Immunogenicity endpoints (secondary objective) were proportion of subjects with any immune responses determined by ELISA and PRNT. A response was defined as either the appearance of antibody titers ≥ assay lower limit of detection (LLOD) for seronegative subjects at baseline or an increase of the antibody titer compared to the baseline titer for subjects with a pre-existing vaccinia specific antibody titer.

Studies POX-MVA-005/023 and POX-MVA-011 used (b) (4) of PRNT, and Studies POX-MVA-008 and POX-MVA-024 used (b) (4) of PRNT. (b) (4) of PRNT used in these studies were insufficiently validated and were not accepted by CBER assay reviewers. The PRNT assay issue precluded us from making any conclusion regarding vaccine effectiveness among the study populations, including for use of a single booster dose in smallpox vaccine-experienced individuals. In addition, the primary endpoints for these studies

were SCR determined by MVA-based ELISA, which is not considered clinically meaningful for inferring vaccine effectiveness. Therefore, the data obtained from these studies were not sufficient to support vaccine effectiveness of two doses of MVA-BN specifically in HIV-infected individuals or individuals with AD subjects, nor to support licensure of a single dose (or to inform timing of a single booster dose) in individuals previously vaccinated with a smallpox vaccine.

However, it was reasonable to conclude that the 2-dose regimen of MVA-BN would be as effective in smallpox vaccine experienced individuals as compared to smallpox vaccine naïve individuals, so smallpox vaccine experienced individuals were included in the approved indication for the 2-dose regimen. Similarly, there is no physiologic reason to suspect decreased effectiveness of MVA-BN in individuals with AD, and benefit-risk of MVA-BN may still be favorable in HIV-infected individuals. Therefore, there is no reason to specifically exclude individuals with AD or infected with HIV from the general indication for use of this vaccine.

Integrated Safety Results

Safety of MVA-BN was assessed in more than 7800 subjects who received at least one dose of MVA-BN in 22 studies under the drug development program. Solicited adverse reactions were collected via diary card for 7 to 14 days after each vaccination, and SAEs and AESIs were followed for at least 6 months after the last vaccination. Across all 22 clinical trials and in all populations including HIV-infected subjects and AD subjects, the safety profile of MVA-BN was favorable.

In a Tris-buffered saline placebo-controlled study, the most commonly reported adverse reactions following any vaccination with MVA-BN were in the System Organ Class (SOC) General Disorders (myalgia 42.8%, headache 34.8%, fatigue 30.4%, nausea 17.3% and chills 10.4%) and Administration Site Conditions (injection-site pain 84.9%, erythema 60.8%, swelling 51.6%, induration 45.4% and pruritus 431.3%). In comparison, the rates of the corresponding adverse reactions reported in the placebo group ranged from 4.6% to 20.5%. Frequencies of adverse reactions were generally similar across the integrated study population. The most commonly reported adverse reactions following MVA-BN vaccination are comparable to other licensed vaccines administered via the SC route.

No clinically relevant difference in the safety and reactogenicity of MVA-BN was observed between vaccinia-naïve and vaccinia-experienced populations. Although there were differences noted in the individual studies, no clear patterns emerged regarding the number and nature of AEs among the different doses and formulations.

Because of the risk of myopericarditis associated with ACAM2000, cardiac adverse events of special interest (AESIs) were monitored during clinical development of MVA-BN. Evaluation of cardiac AESIs included any cardiac signs or symptoms, ECG changes determined to be clinically significant, or troponin-I elevated 2 times the upper limit of normal (ULN). In the 22 studies, subjects were monitored for cardiac-related signs or symptoms through at least 6 months after the last vaccination.

The numbers of MVA-BN and placebo recipients, respectively, with troponin-I data were: baseline level (6,376 and 1,203); level two weeks after first dose (6,279 and 1,166); level two weeks after second dose (1,683 and 193); unscheduled visit, including for clinical evaluation of suspected cardiac adverse events (500 and 60).

Cardiac AESIs were reported to occur in 1.3% (95/7,093) of MVA-BN recipients and 0.2% (3/1,206) of placebo recipients who were smallpox vaccine-naïve. Cardiac AESIs were reported to occur in 2.1% (16/766) of MVA-BN recipients who were smallpox vaccine-experienced. The higher proportion of MVA-BN recipients who experienced cardiac AESIs was driven by 28 cases of asymptomatic post-vaccination elevation of troponin-I in two studies: POX-MVA-011, which enrolled 482 HIV-infected subjects and 97 healthy subjects, and POX-MVA-008, which enrolled 350 subjects with atopic dermatitis and 282 healthy subjects. An additional 127 of asymptomatic post-vaccination elevation of troponin-I above the ULN but not above 2 times the ULN were documented in MVA-BN recipients throughout the clinical development program, 124 of which occurred in studies POX-MVA-011 and POX-MVA-008. Proportions of subjects with troponin-I elevations (> ULN) were similar between healthy (13.7%) and HIV-infected (11.5%) subjects in POX-MVA-011 and between healthy (18.9%) and atopic dermatitis (18.0%) subjects in POX-MVA-008.

Overall, the number of subjects with AESIs in this clinical development program was relatively low. Except for one case of suspected pericarditis that was assessed as unlikely related to MVA-BN and isolated mild to moderate increases of troponin levels with unknown clinical significance, there were no other reported cardiac AESIs. Among the 22 studies, all the studies except for studies POX-MVA-008 and POX-MVA-011 had few subjects with post-vaccination elevation of troponin-I. The applicant postulates that the increased proportion of subjects with post-vaccination elevation of troponin-I is related to the use of a more sensitive troponin assay. Among these two studies, 188 subjects were assessed for troponin-I with a ”conventional” troponin assay, and 934 subjects were assessed for troponin-I with a “high sensitivity” troponin assay. The “high sensitivity” troponin assay used in these two studies was not cleared by FDA. Among the 188 subjects whose troponin-I was assessed with the “conventional” troponin assay, no subject reported post-vaccination elevation of troponin-I, while 144 out of 934 subjects whose troponin-I was assessed by the “high sensitivity” troponin assay reported post-vaccination troponin-I elevation. All subjects with

elevated troponin-I levels underwent a cardiologist workup and no clinically meaningful cardiac abnormality was identified among these subjects. Since there was no placebo control in these two studies, the clinical relevance of the increased proportion of subjects with subclinical, yet abnormal troponin-I is unknown.

Across the 22 clinical trials, no trends for unexpected and/or serious adverse events due to the investigational product were detected.

In addition, none of the historically reported complications of replicating vaccinia-based smallpox vaccines, such as vaccinia rash, eczema vaccinatum, generalized vaccinia, progressive vaccinia, erythema multiforme or post-vaccinal encephalitis have been observed in the clinical development program of MVA-BN.

There were two deaths reported from the 22 clinical trials: one each was reported from POX-MVA-011 (due to overdose of Xanax and benzodiazepine) and POX-MVA-013 (suicide), respectively. None was deemed related to MVA-BN by the investigator, applicant or clinical reviewer.

Clinical Lot Consistency Study

POX-MVA-013 was a randomized, double-blind, placebo-controlled Phase 3 lot consistency and safety study in healthy, vaccinia naïve subjects. Approximately 4000 subjects were randomized into four study groups (1:1:1:1 via block randomization) to receive two doses of 1 of 3 MVA-BN lots or placebo 28 days apart. The primary objective was to assess the consistency of 3 consecutively produced MVA-BN lots. Lot equivalence was pre-specified as 95% CI of PRNT GMT ratio of each two lots between 0.5 and 2.0.

The study enrolled 4005 subjects: 999, 1005 and 999 subjects in 3 MVA-BN lot groups respectively, and 1002 subjects in placebo group. The average age of subjects was 27.7 years of age, 52.1% subjects were females, and 77.4% subjects were white. The demographic distribution among the four groups was similar,

PRNT GMTs at Peak Visit were similar between the 3 MVA-BN lot groups, 110.5 (95% CI: 103.3, 118.1), 100.7 (95% CI: 94.0,107.9), 117.0 (95% CI: 108.9, 125.8) for MVA-BN lot 1, 2, and 3, respectively. The ratios of PRNT GMTs between MVA-BN lots were:

- Lot 2 to 3: 0.86 (95% CI: 0.78, 0.95),
- Lot 1 to 3: 0.94 (95% CI: 0.86, 1.04), and
- Lot 1 to 2: 1.1 (95% CI: 1.00, 1.21).

These ratios fell in the pre-defined range for equivalence of 0.5-2.0. The study demonstrated that the 3 consecutively produced vaccine lots were equivalent.

Pediatric Assessment

The Pediatric Research Equity Act (PREA) (21 U.S.C. 355c) requires the conduct of pediatric studies for certain drug and biological products. The applicant requested a full waiver of the pediatric assessment for MVA-BN because there are no pediatric populations currently at risk of smallpox, and pediatric populations at risk of monkeypox are small and dispersed among deeply forested regions of central and western Africa. Therefore, necessary studies are impossible or highly impracticable, and we granted the request.

Summary of Pharmacovigilance Plan

The clinical reviewers have not identified a safety signal in the submission. However, two clinical trials (POX-MVA-008 and POX-MVA-011) showed a substantial number of study subjects with post-vaccination elevation of troponin-I of uncertain clinical significance and potentially related to an assay issue. The applicant has proposed a post-licensure observational study in the event of a smallpox event where any collected cardiac data will be used to further assess for a cardiac signal.

Conclusion and Regulatory Recommendation

Vaccine effectiveness against smallpox and monkeypox was inferred by comparing the immunogenicity of MVA-BN to a licensed smallpox vaccine (ACAM2000) based on a PRNT using the VV-WR and was supported by efficacy data from animal challenge studies. The submitted data support the effectiveness of a two-dose regimen of MVA-BN in preventing smallpox and monkeypox.

The safety profile of MVA-BN in the study population including HIV infected subjects and subjects with AD or AD history was favorable. The studies did not observe increased cardiac events among MVA-BN vaccinated individuals compared with placebo recipients. The submitted safety data also support use of MVA-BN in subjects such as individuals infected with HIV, or with AD.

In conclusion, this reviewer recommends approval of the proposed indication and 2-dose regimen of MVA-BN in individuals 18 years of age and older.

1.1 Demographic Information: Subgroup Demographics and Analysis Summary

Subgroup Analysis of Effectiveness

In the pivotal effectiveness trial, POX-MVA-006, all subgroups (stratified by age, sex, race and ethnicity) except one (American Indian or Alaskan Native subgroup), vaccinia specific neutralizing antibody titers measured by PRNT GMTs at Peak Visits among MVA-BN vaccinated subjects (Group 1) were non-inferior to those among subjects vaccinated with ACAM2000 (Group 2). The LBs

of 95% CI for the corresponding GMT ratios (Group 1/Group 2) at Peak Visit were >0.5, the pre-specified non-inferiority margin for the primary endpoint of immunogenicity.

For the subgroup of American Indian or Alaskan Native, the PRNT GMT at Peak Visit among MVA-BN vaccinated subjects was lower than that among ACAM2000 vaccinated subjects. The clinical significance is unknown due to the limited number of subjects (n=7 in Group 1, and n=4 in Group 2) in this subgroup.

Subgroup Analysis of Safety

Subgroup analysis of solicited adverse reactions was conducted on the Main ISS population pooled from 12 clinical trials that assessed the safety of the to-be-licensed vaccination regimen. Subgroup analyses of AESI and SAE were conducted on the broad ISS population including all 22 clinical trials in the product development program.

Subpopulation Analyses of Solicited Systemic Reactions

Among the vaccinia-naïve subjects vaccinated with MVA-BN, percentages of subjects with solicited systemic reactions were similar among different ages (18 to 40 years of age vs. >40 years of age), and ethnicities (Hispanic/Latino vs. Non-Hispanic/Latino vs. others). More female subjects (64.4%) reported solicited systemic reactions compared with male subjects (51.8%). Percentages of subjects with solicited systemic reactions among American Indian/Alaska Native (62.5%), white (60.2%) and other/not reported (63.7%) subjects were numerically higher than those among Asian (45.8%), black/African (48.6%) and Native Hawaiian or Other Pacific Islander (47.6%) subjects.

Among vaccinia-experienced subjects vaccinated with MVA-BN, 404 of 409 subjects were white, and there were no Hispanic/Latino subjects in this population. More female subjects (54.6%) experienced solicited systemic reactions compared with male subjects (46.2%), and more subjects >55 years (89.5%) reported solicited systemic reactions compared with subjects 18 to 55 years of age (52.6%).

Subpopulation Analyses of Solicited Injection-Site Reactions

Among the vaccinia-naïve subjects vaccinated with MVA-BN, percentages of subjects with solicited injection-site reactions were similar between different ethnicity groups (ranging from 83% to 88%). More female subjects (92.1%) reported solicited injection-site reactions compared with male subjects (81.7%), and more subjects 18 to 40 years of age (87%) experienced injection-site reactions compared with subjects >40 years of age (78%). Percentages of subjects with solicited injection-site reactions among Asian (76.5%) and black/African American (76.9%) subjects were numerically lower compared with

white (89.6%), American Indian/Alaska Native (87.5%), other/not reported (87.1%) and Native Hawaiian or Other Pacific Islander (85.7%) subjects.

Among vaccinia-experienced subjects vaccinated with MVA-BN, percentage of subjects with solicited injection-site reactions were similar between males and females. More subjects 18 to 55 years (95.5%) reported solicited injection-site reactions compared with subjects > 55 years of age (80.7%).

Subgroup Analyses of Cardiac AESIs in the ISS Population

For vaccinia-naïve healthy and vaccinia-experienced healthy subjects, the proportion of subjects with AESIs was higher in the older age group [> 40 years-old for vaccinia-naïve subjects, 5.7% (3/53) and >55 years-old for vaccinia-experienced [4.1% (5/121)] compared to the subjects in the younger age group [18–40 years-old vaccinia-naïve subjects, 0.8% (52/6213), or 18-55 years-old vaccinia-experienced subjects, 1.9% (8/411)], whereas the opposite was true for vaccinia-naïve AD subjects [5.8% (22/380) among 18-40 years vs. 0% (0/1) among >40 years] or HIV-infected subjects [3.8% (13/341) among 18-40 years vs. 2.9% (4/137) among >40 years].

The most frequently reported cardiac AESI was post vaccination elevation of troponin-I. Post vaccination elevation of troponin-I reported in the vaccine development program was concentrated in two studies (POX-MVA-011 and POX-MVA-008) in which most of subjects' troponin-I were assessed with a "high sensitivity" assay that was not clearly by FDA. Higher rates of subjects with post-vaccination elevation of troponin-I were likely attributed to the troponin assay. The clinical significance of asymptomatic troponin elevations was unknown.

No obvious difference was observed among male and female subjects, or among different race and ethnicity subgroups.

For vaccinia-naïve AD or HIV infected subjects, the proportion of subjects with AESIs was slightly higher in the Black/African American race group [17.6% (6/34) among AD subjects, and 3.3% (6/180) among HIV infected subjects) compared with the other race groups (0% to 9.4% among AD subjects, and 0% to 2.7% among HIV infected subjects). No other differences were observed when the data were stratified by race or ethnicity. Also, when displayed by treatment group, there were no relevant differences in the stratifications for race and ethnicity between the analyzed groups, neither for overall incidence nor with regard to the pattern of reported AESIs.

Subgroup Analyses of SAEs

For MVA-BN vaccinated vaccinia-naïve populations, there was no apparent pattern or meaningful difference in overall SAE frequencies among subpopulations stratified by sex, race and ethnicity. The subgroup of > 40-year-

olds had a numerically higher rate of SAEs [2.6% (5 out of 191 subjects)]. However, all 5 SAEs reported by this subgroup were from subjects infected with HIV and none of the SAEs were assessed as treatment related.

For MVA-BN vaccinated, vaccinia-experienced subjects, the overall SAE frequencies varied greatly among the subgroups except for the ethnicity subgroup due to the limited number of SAEs and small study sample size. The numerically higher percentages of subjects with SAEs in the subgroups of males [2.9% (12/147) vs. 1.7% (6/349) among females] and >55 year olds [4.8% (6/126) vs. 1.9% (12/640) among subjects 18-55 years] were primarily driven by three cases of prostate cancer in these subgroups. The apparently higher percentage of SAEs in the black subgroup was likely a random effect, and none of these SAEs (one case each of cellulitis, hip arthroplasty, and pneumothorax, and two cases of gastroenteritis) was deemed related to the treatment by the applicant or the clinical reviewer.

1.2 Patient Experience Data

Patient experience data were not submitted to this BLA.

2. CLINICAL AND REGULATORY BACKGROUND

2.1 Disease or Health-Related Condition(s) Studied

Smallpox is a highly contagious infectious disease caused by either of two closely related viruses, variola major and variola minor. Variola virus belongs to the genus *Orthopoxvirus*, family Poxviridae. Poxviruses are large brick-shaped viruses with a double stranded DNA genome. Transmission of variola viruses generally requires direct and fairly prolonged face-to-face contact between people. Smallpox also can be spread through direct contact with infected bodily fluids or contaminated objects such as bedding or clothing. Rarely, smallpox has been spread by virus carried in the air in enclosed settings such as buildings, buses, and trains. Humans are the only natural hosts of variola. Smallpox is not known to be transmitted by insects or animals, and there is no asymptomatic carrier state[1].

The incubation period of smallpox averages 12 days, with a range of 7 to 17 days. A two to three day prodrome of high fever, malaise, and prostration with headache and backache is followed by the development of maculopapular rash. Within one to two days, the rash becomes vesicular and then pustular. The pustules are characteristically round, tense, and deeply embedded in the dermis. Crusts begin to form about the eighth or ninth day. When the crusts separate, they leave pigment-free skin and frequently pitted scars. Death usually occurs late in the first week or during the second week of the illness and is probably due to the effects of an overwhelming viremia[2].

There are two clinical forms of smallpox. Variola major, caused by variola major virus, is the severe and most common form of smallpox, with a more extensive rash and higher fever. Variola minor is a less common presentation of smallpox, and a much less severe disease, with death rates historically of 1% or less. There are four types of variola major smallpox: 1) ordinary (the most frequent type, accounting for 90%), 2) flat (also known as “malignant”), 3) hemorrhagic and 4) modified (mild and occurring in previously vaccinated persons). Utilizing this classification, Rao AR analyzed the incidence and mortality of vaccinated and unvaccinated patients in Madras India[1]. Among those not previously vaccinated, the vast majority of variola major cases (approximately 90%) were of the ordinary type, with a mortality of approximately 30%. Flat smallpox accounted for 6.7% of the cases and had a mortality of 96%. Hemorrhagic smallpox accounted for 2.4% with a mortality of 96%. Modified cases had many fewer lesions, accounted for 2.1%, and resulted in no death.

Smallpox outbreaks have occurred from time to time for thousands of years, but the disease is now eradicated after a successful worldwide vaccination program. The last case of smallpox in the United States was in 1949. The last naturally occurring case in the world was in Somalia in 1977. After the disease was eliminated from the world, routine vaccination against smallpox among the general public was stopped because it was no longer necessary for prevention.

Despite the fact that the World Health Organization (WHO) officially declared successful global eradication of smallpox in 1980, the existence of variola stockpiles and the threat of bioterrorism makes maintaining immunity to smallpox through vaccination critical. After the events of September 11th, 2001, concern over the use of bioweapons as agents of terrorism increased[3]. As mass vaccination programs halted more than 30 years ago, it is estimated that the majority of the world population has no existing immunity to smallpox, and as such, the release of this highly contagious virus would have devastating effects. Although ACAM2000 was approved for prevention of smallpox, it is associated with severe adverse events as described in Section 2.3 of this review and is consequently contraindicated in individuals with severe immunodeficiency who are not expected to benefit from the vaccine. Due to its safety risks, ACAM2000 has been used primarily in US military personnel whose deployment has been determined by the US government to be associated with increased risk of smallpox bioterrorism exposure. As a consequence, an urgent need exists for a safer and efficacious vaccine to protect the public against smallpox.

Monkeypox is a rare viral zoonotic disease that occurs primarily in remote parts of central and west Africa, near tropical rainforests. The monkeypox virus (MPXV) is primarily transmitted to people from various wild animals such as rodents and primates but has limited secondary spread through human-to-human transmission. MPXV is similar to variola smallpox, and the clinical presentation following MPXV infection is also similar to smallpox. Although monkeypox is much milder than smallpox, it can be fatal. Typically, case fatality in monkeypox

outbreaks has been between 1% and 10%, with most deaths occurring in younger age groups.

Human monkeypox was first identified in humans in 1970 in the Democratic Republic of Congo in a 9 -year-old boy. Since then, human cases of monkeypox have been reported from 10 African countries – Democratic Republic of the Congo, Republic of the Congo, Cameroon, Central African Republic, Nigeria, Ivory Coast, Liberia, Sierra Leone, Gabon and South Sudan.

In the spring of 2003, monkeypox cases were confirmed in the United States of America, marking the first reported occurrence of the disease outside of the African continent. Most of the patients were reported to have had close contact with pet prairie dogs that were infected by African rodents that had been imported into the country. The most current outbreak of monkeypox occurred in Nigeria in 2017[4].

2.2 Currently Available, Pharmacologically Unrelated treatment/Intervention for the Proposed Indications

On July 13, 2018, the U.S. Food and Drug Administration approved TPOXX (tecovirimat) under FDA's Animal Rule pathway, the first drug with an indication for treatment of smallpox. TPOXX's effectiveness against smallpox was established by studies conducted in animals infected with viruses (monkeypox for non-human primates and rabbitpox for rabbits) that are closely related to the virus that causes smallpox and was based on measuring survival at the end of the studies. More animals treated with TPOXX lived compared to the animals treated with placebo. Only one monkey in the placebo group survived monkeypox virus challenging among the four pivotal monkey animal studies, and no rabbit in placebo group survived rabbitpox virus challenge in the placebo controlled pivotal rabbit animal study.

Efficacy of TPOXX (ranging from 50% to 100%) is dose dependent and is also dependent on initial treatment time point following infection. The safety of TPOXX was evaluated in 359 healthy human volunteers without a smallpox infection. Only one treatment unrelated serious adverse event (i.e., pulmonary embolism) was reported in TPOXX group. The most frequently reported side effects were headache, nausea and abdominal pain.

Reviewer's comments: *The clinical benefit of TPOXX has not been verified in humans. Some uncertainties exist regarding its effectiveness in humans. Although TPOXX may be efficacious in treating smallpox disease or preventing it from getting worse, vaccination is still the only historically proven effective intervention to prevent this deadly disease.*

There is no specific treatment or vaccine available for monkeypox. Although ACIP recommends ACAM2000 for prevention of monkeypox in individuals at high

risk of exposure (e.g., lab workers who handle monkeypox virus) it is not US-licensed for this use.

2.3 Safety and Efficacy of Pharmacologically Related Products

During the smallpox endemic era, replicating smallpox vaccines were based on several different vaccinia virus (VV) strains, such as Dryvax (a New York City Board of Health strain) used in the United States (US) and in Canada[5] and the Lister-Elstree strain used primarily in Europe. These vaccines were proven to be highly effective but have also been associated with severe and occasionally life-threatening adverse events, especially in children, individuals with congenital or acquired immune deficiency disorders, individuals with chronic exfoliative skin conditions, and pregnant women[6]. Manufacture of these vaccines was suspended after smallpox was declared officially eradicated in 1980 by WHO.

Currently, ACAM 2000 is the only FDA-licensed vaccine for active immunization against smallpox for individuals determined to be at high risk for contracting the disease. ACAM2000 is a live, replication-competent vaccinia virus vaccine and was cloned from Dryvax.

ACAM2000 was approved in August 2007 based on non-inferiority comparison of co-primary endpoints, take rates and anti-vaccinia neutralizing antibody titers, with Dryvax in both vaccinia-naïve and -experienced populations. In vaccinia-naïve populations, ACAM2000 was demonstrated to be non-inferior to Dryvax based on the take rates. However, ACAM2000 did not meet the protocol pre-specified non-inferiority criterion for vaccinia neutralizing antibody titers. In vaccinia-experienced populations, ACAM2000 was demonstrated to be non-inferior to Dryvax based on vaccinia neutralizing antibody titers but did not meet the protocol pre-specified non-inferiority criterion for take rates.

ACAM2000 can cause serious adverse events (SAEs) in vaccinees and their close contacts, especially in high-risk populations such as immunodeficient individuals. As indicated in the boxed warning of the ACAM2000 package insert (PI), myocarditis and pericarditis, encephalitis, encephalomyelitis, encephalopathy, progressive vaccinia, generalized vaccinia, severe vaccinia skin infections, erythema multiforme major (including Stevens-Johnson syndrome), eczema vaccinatum resulting in permanent sequelae or death, ocular complications, blindness, and fetal death have occurred following either primary vaccination or revaccination with live vaccinia virus smallpox vaccines. These risks are increased in certain individuals and may result in severe disability, permanent neurological sequelae, and/or death.

Reviewer's comments: *Per the PI of ACAM2000, suspect cases of myocarditis and pericarditis were observed at a rate of 5.7 (95% CI: 1.9-13.3) per 1,000 primary vaccines (5 of 873 primary vaccines in the Phase 3 trial). Of the 5 suspect cases of myopericarditis, 3 cases were subclinical (all were associated with clinically significant ECG changes) and 2 cases were symptomatic, and all of*

them were observed in the pivotal phase 3 trial in the vaccinia-naïve population and all were resolved. Two additional suspect cases of myopericarditis (one subclinical and one symptomatic) were identified in vaccinia-naïve subjects treated with ACAM2000 in a Phase 1 and a Phase 2 trial respectively. Across the ACAM2000 clinical program, a total of 7 cases of myocarditis/ myopericarditis were identified in 2,893 ACAM2000 treated subjects.

2.4 Previous Human Experience with the Product (Including Foreign Experience)

MVA-BN, in liquid-frozen (LF) formulation, was licensed under the invented name IMVANEX in the European Union under exceptional circumstances via the centralized procedure in July 2013. The same formulation of MVA-BN was licensed in Canada as an Extraordinary Use New Drug with proprietary name IMVAMUNE in November 2013.

In the US, the Centers for Disease Control and prevention (CDC) submitted clinical data generated in populations at greatest risk for complications from replicating smallpox vaccines to support the use of MVA-BN for individuals with HIV infection or atopic dermatitis (AD) of all ages in the event that a smallpox public health emergency is declared in the U.S.

To date no post-marketing data exists for MVA-BN.

2.5 Summary of Pre- and Post-submission Regulatory Activity Related to the Submission

Pre-submission Regulatory Activities

30 Nov. 2011 FDA and BN held a teleconference to discuss licensure pathway and the meeting concluded that the most appropriate approach to licensure for MVA-BN under the traditional approval pathway would be to demonstrate immunologic non-inferiority to ACAM2000 in a clinical trial. Accordingly, a Phase 3 non-inferiority trial, POX-MVA-006, was designed with a primary endpoint of neutralizing antibody titers determined by plaque reduction neutralization test (PRNT).
FDA further advised that this study include, at a minimum, variola *in vitro* neutralization with representative samples from the pivotal clinical trial and animal model studies which provide additional reassurance of efficacy. FDA emphasized that this licensure approach would not invoke the Animal Rule (21 CFR 601.90). FDA recommended that animal protection studies be conducted in at least two animal models, those being a non-human primate monkeypox virus (MPXV) and mouse ectromelia (ECTV), to provide support of effectiveness of the product.

Reviewer's comment: *Since in vitro neutralization assays with variola virus must be conducted in a biosafety level 4-certified laboratory with access to variola virus, the applicant had to rely on the US Centers for Disease Control and Prevention (CDC) to conduct these experiments. CDC was not able to conduct the experiments using serum samples from the pivotal clinical trial, POX-MVA-006, prior to the BLA being ready for submission, and we determined that variola neutralization data would not be necessary to support approval of the BLA.*

12 Sept. 2013 FDA commented on the proposed pivotal study POX-MVA-006: BN proposed to replace immunologic non-inferiority to ACAM2000 with take attenuation following ACAM2000 in MVA-vaccinated subjects vs. smallpox vaccine naïve subjects as the primary endpoint for POX-MVA-006. The applicant proposed that take attenuation provided clinically meaningful evidence that vaccinia virus replication at the ACAM2000 inoculation site was suppressed by the immune response to MVA-BN. FDA acknowledged that prior variola infection or prior smallpox vaccination could result in attenuation of the "take" reaction. However, the predictive relationship between attenuation of this reaction and protection against smallpox disease has not been sufficiently well established to support attenuation of "take" as a surrogate primary efficacy endpoint. Therefore, FDA did not concur with the proposed "take" attenuation as the primary endpoint for licensure. Neutralizing antibody titers, while not detectable by currently validated methods early in the post-vaccination period, are likely to be predictive of protective immunity when measured at peak time points and have regulatory precedence for supporting efficacy of smallpox vaccines. Consequently, FDA recommended that neutralizing antibody GMT at the pre-specified peak time points remain the primary endpoint for this study, and that "take" attenuation be included as a supportive secondary endpoint. BN then proposed to use vaccinia neutralizing antibody and take attenuation as co-primary endpoints, and FDA accepted the proposal.

15 Jan. 2016 End of Phase 2 (EoP2) meeting: BN anticipated submitting MVA-BN LF (b) (4) formulations in a single BLA filing. However, due to availability of data from the pivotal Phase 3 non-inferiority trial, POX-MVA-006, the initial BLA would be based on the LF formulation only and the (b) (4) .

29 Jun. 2017 Type C meeting: BN received feedback regarding the proposed content of the nonclinical and clinical modules of the BLA.

30 May 2018 pre-BLA meeting: FDA provided responses to specific questions raised by BN regarding the overall format and content of the administrative, nonclinical, and clinical sections of the BLA for MVA-BN.

Reviewer's comment: *Studies POX-MVA-005 and -023 (please refer to Section 6.2 of this review) were conducted prior to our EOP-2 meeting with the applicant. The applicant did not propose until the pre-BLA meeting that these two studies would be intended as the primary basis to support use of a single booster dose in subjects previously vaccinated with smallpox vaccine. Following the pre-BLA meeting we agreed to consider the data from these studies in our review of the licensure application and provided a preliminary opinion, pending full review of the data and assay validation package to be submitted in the BLA, that the data would support the proposed booster dose.*

3. SUBMISSION QUALITY AND GOOD CLINICAL PRACTICES

3.1 Submission Quality and Completeness

Although the submission was well organized, the ISS consisted only of tables without an in-depth analysis of all clinical safety data. The dataset for the ISS did not include safety information from the comparator groups nor did it include demographic information. No subgroup analyses by age, race, sex, or ethnicity were provided for the safety or effectiveness data.

Injection-site reactions and vaccination-site reactions, which are synonymous, were used in the submission simultaneously. Some solicited injection-site reactions were excluded from the solicited adverse event dataset and were included in the dataset of unsolicited events.

Also a number of other errors and omissions in the submission necessitated multiple requests for information and clarifications and teleconferences with the applicant.

Reviewer's comment: *We received responses to all the information requests (IRs). The majority of responses were satisfactory, and those that were not are detailed throughout the review.*

3.2 Compliance With Good Clinical Practices And Submission Integrity

In the Clinical Overview, the applicant states that all trials were approved by the competent Ethics Committee (EC)/Institutional Review Board (IRB) according to the national and local laws of the respective sites before the first subject was screened, and all clinical trials were performed in complete accordance with the provisions of the Declaration of Helsinki (and its respective amendments), the national laws, and other guidelines for the conduct of clinical studies, such as the ICH Harmonized Tripartite Guideline for Good Clinical Practice (GCP).

3.3 Financial Disclosures

The applicant made reasonable efforts to obtain financial disclosure from all investigators and sub-investigators who participated in the covered studies as defined in 21 CFR 54.2(e) submitted to the BLA.

In the Financial Certification and Disclosure Form, the applicant listed all the investigators in the covered studies, and certified that no financial arrangements with an investigator had been made where study outcome could affect compensation; that the investigator had no proprietary interest in the tested product; that the investigator did not have a significant equity interest in the sponsor of the covered study; and that the investigator had not received significant payments or other sorts. The applicant also certified that complete certification or disclosure was obtained for all investigators in the covered clinical studies, and that the applicant was not aware of any investigators in the covered clinical trials with disclosable financial arrangements.

4. SIGNIFICANT EFFICACY/SAFETY ISSUES RELATED TO OTHER REVIEW DISCIPLINES

4.1 Chemistry, Manufacturing, and Controls

The product reviewer did not identify any significant CMC issue. Please refer to the product reviewer's review for details.

4.2 Assay Validation

The only issue identified by the assay validation reviewer is the PRNT assay validation on the determination of the LLOQ and the data to show the assay accuracy near the LLOQ range.

(b) (4) versions of PRNT were used among the different trials under the vaccine development program. (b) (4) versions of PRNT (Versions (b) (4)) were validated and accepted by CBER assay reviewers. These (b) (4) versions were used in POX-MVA-013 (Version (b) (4)) and POX-MVA-006 (Version (b) (4)).

The other (b) (4) versions, Versions (b) (4), could not be validated by CBER assay reviewers due to the expiration of the agents used in the assay. The immunogenicity data derived from these (b) (4) versions of PRNT were reviewed but will not be presented in the package insert. The impacted studies include POX-MVA-005, -023, and -011 (Version (b) (4)), and POX-MVA-008 and -024 (Version (b) (4)).

Please refer to the assay reviewer's review for details.

4.3 Nonclinical Pharmacology/Toxicology

General toxicology:

Four general toxicology studies were submitted to support this BLA. All studies were repeat dose studies. The toxicology reviewer states that there are no significant safety issues to preclude the BLA from approval.

Reproductive studies:

In four developmental toxicity studies, the effect of MVA-BN on embryo-fetal and post-natal development was evaluated in pregnant rabbits and rats. No vaccine-related fetal malformation or variations and adverse effects on pre-weaning development were reported in these studies.

Genotoxicology studies:

No genotoxicology studies were submitted to this BLA.

Please refer to the toxicology reviewer's review for details.

Non-human primate challenge studies:

The efficacy of MVA-BN to protect cynomolgus macaques against a MPXV challenge was evaluated in several studies. Animals were administered Tris-Buffered Saline (placebo) or MVA-BN (1x 10^8 $TCID_{50}$) subcutaneously (SC) on day 0 and day 28. On day 63, animals were challenged with MPXV delivered by aerosol (3 x 10^5 pfu), intravenous (5 x 10^7 pfu) or intratracheal (5 x 10^6 pfu) route. Across all studies, 80-100% of MVA-BN-vaccinated animals survived compared to 0-40% of control animals.

Mouse challenge studies:

The protective efficacy of MVA-BN was evaluated in several studies conducted with a murine intranasal (i.n.) challenge model based on ECTV. (b) (4) mice were treated with either placebo (Tris-Buffered Saline) or MVA-BN (1x10^8 $TCID_{50}$) given subcutaneously on Day 0 and Day 28. On Day 42, the treated mice were challenged with a lethal i.n. dose of ECTV of 58x MLD_{50}. All immunized mice survived the lethal challenge with ECTV and all mice in the placebo groups succumbed to the viral challenge (as shown by signs of sickness on Day 6 and death occurring on Day 7 – 8).

Please refer to the product review for details.

4.4 Clinical Pharmacology

4.4.1 Mechanism of Action

Vaccinia virus is a member of the same taxonomic group (the Orthopox genus) as smallpox (variola) virus and MPXV, and immunity induced by vaccinia virus

cross-protects against variola virus and MPXV. Replicating vaccinia virus causes a localized virus infection and inflammation at the site of inoculation forming a pustular skin lesion generally referred to as a "take" which provides evidence of protective immunity. Vaccination with vaccinia virus based vaccines induces neutralizing antibodies as well as T-cell responses. Anti-vaccinia neutralizing antibody is believed to be a correlate of product against smallpox[7]; however, the level of neutralizing antibody that protects against smallpox has not been established.

Reviewer's comment: *Since MVA-BN is non-replicating virus, it does not induce a take reaction, which is the reason why rates of take reactions could not be directly compared between MVA-BN and ACAM2000.*

4.5 Statistical

The statistical reviewer verified the results of the applicant's analyses of the immunogenicity and safety data. Please refer to the CBER statistical reviewer's memo for details.

4.6 Pharmacovigilance

Although no safety signal regarding cardiac events has been identified from the studies, up to 18.4% of subjects in two of the 22 clinical trials were reported to have abnormal, asymptomatic troponin-I elevations following vaccination. These troponin-I elevations were not accompanied by clinically significant ECG changes or other findings on cardiology evaluation and were of uncertain clinical significance. We find the applicant's proposed plan to assess spontaneously reported cardiac data as part of the routine post-marketing pharmacovigilance plan acceptable. Please refer to the pharmacovigilance reviewer's review for detail.

5. Sources of Clinical Data and Other Information Considered in the Review

5.1 Review Strategy

General Review Strategy

The applicant, Bavarian Nordic (BN), submitted a total of 22 clinical studies evaluating the safety and immunogenicity of MVA-BN including its different dosages, administration routes, and schedules, and formulations. Of them, 12 studies employed the two dose regimen (two doses of MVA-BN in LF formulation, each at 0.5 mL, administered SC at 28 days apart) for vaccinia-naïve populations, as well as a single booster dose in smallpox vaccine experienced individuals. Among these 12 studies, 7 studies are considered essential to support the proposed indication and usage and are reviewed in detail and documented in Section 6 (POX-MVA-006, -005/023, and -013) and Section 9

(Section 9.1.4: POX-MVA-011, Section 9.1.5L POX-MVA-024, and Section 9.2: POX-MVA-008) of this review.

- Study POX-MVA-006 provides immunogenicity in comparison with licensed smallpox vaccine ACAM2000 as well as safety data to support licensure of MVA-BN in smallpox vaccine naïve population.
- Study POX-MVA-005 and its extension study POX-MVA-023 provide immunogenicity and safety of MVA-BN in a smallpox vaccine experienced population.
- Study POX-MVA-013 provides lot consistency data of three consecutively manufactured lots of MVA-BA as well as additional safety data of MVA-BN.
- Studies POX-MVA-008, POX-MVA-11, and POX-MVA-024 provide safety and limited immunogenicity data in subjects with atopic dermatitis, in HIV infected subjects, and in vaccinia-experienced healthy subjects 56 through 80 years of age, respectively.

In addition, studies POX-MVA-004 and POX-MVA-002 evaluated pharmacodynamics of MVA-BN in (b) (4) formulation. These studies provided scientific foundation for selection of an effective dose and regimen for late phase studies. Therefore, studies POX-MVA-002 and -004 were also reviewed and documented in the Appendix.

Reviewer's comment: *No dose ranging study with the LF formulation of MVA-BN was conducted under the drug development program. However, two studies, POX-MVA-027 and -029, demonstrated that the safety profile of MVA-BN (b) (4) and MVA-BN LF were similar, and vaccinia specific neutralizing antibodies elicited by the (b) (4) formulations were equivalent. It is acceptable to use the dose ranging data obtained from the (b) (4) formulation to support the dose selection of the LF formation.*

The data regarding a booster dose regimen did not support its approval due to assay issues. Please refer to Section 6.2 for details.

Since only one effectiveness study with an active comparator (POX-MVA-006) exists, and vaccinia specific neutralizing antibody titers determined by PRNT vary greatly across studies, we concurred with the applicant that an integrated summary of efficacy (ISE) is not required.

The integrated summary of safety (ISS) is assessed in two datasets: the Main ISS is pooled from 12 clinical trials that assessed safety of the to-be-licensed regimen and formulation and the broader ISS (referred to as the ISS in this review) is pooled from all 22 clinical trials. The broader ISS focuses on SAEs and cardiac adverse events of special interest (AESIs) only. Both the Main ISS and the broader ISS are documented in Section 8.

Joint Review Responsibilities

Dr. Sixun Yang reviewed the two pivotal studies, POX-MVA-006 in vaccinia-naïve subjects and POX-MVA-005 in vaccinia-experienced subjects, including its extension study, POX-MVA-023, and the dose ranging studies (POX-MVA-001, POX-MVA-002, and POX-MVA-004), as well as the integrated summaries of safety. Dr. Yang also assumed responsibilities for synthesis and documentation of the overall conclusions and the executive summary for the application.

Dr. Alexandra Yonts reviewed the lot consistency clinical trial, POX-MVA-013, which was the largest placebo controlled clinical trial and also served as the basis for solicited adverse reaction data in smallpox naïve individuals for product labeling. Dr. Yonts also reviewed the following clinical trials in special populations: POX-MVA-008 in AD subjects, POX-MVA-011 in HIV infected subjects and POX-MVA-024 in vaccinia-experienced healthy subjects 56 through 80 years of age. Additionally, Dr. Yonts played a primary role in evaluating and reconciling discrepancies in troponin-I elevation reports and cardiac AESIs across the major studies included in this review.

5.2 BLA/IND Documents That Serve as the Basis for the Clinical Review

This clinical review considered the following documents submitted to the BLA, as listed by electronic common technical document (eCTD) module:

- STN125678/0, Module 1.3 (Financial Certification and Disclosure)
- STN125678/0, Module 1.9 (Pediatric Assessment Plan)
- STN125678/0, Module 1.14 (Labeling)
- STN125678/0, Modules 2.2, 2.4, 2.5 and 2.7 (Introduction, Nonclinical summary, Clinical Overview, and Clinical Summary)
- STN125678/0, Module 5.3.4 (Pharmacodynamic [i.e., Dose Ranging] Studies)
- STN125678/0, Module 5.3.5.1 (Study Reports of Controlled Clinical Studies Pertinent to the Claimed Indication)
- STN125678/0, Module 5.3.5.2 (Study Reports of Uncontrolled Clinical Studies)
- STN125678/0, Module 5.3.5.3 (Reports of Analyses of Data from More than One Study, i.e., Integrated Summary of Safety)
- STN125678/0, Module 5.3.5.4 (Other Study Reports)
- STN125678/0.7, Module 1.11.3 (Responses to CBER's IR #3 regarding ISS Presentation)
- STN125678/0.9, Module 1.11.3 (Responses to CBER's IR #8 regarding Take Rates and Cut-off Values for PRNT and ELISA)
- STN125678/0.13, Module 1.11.3 (Responses to CBER's IR #8 regarding Troponin Assay)
- STN125678/0.14, Module 1.11.3 (Responses to CBER's IR #10 regarding Data Validation and Subgroup Analysis of Main ISS)
- STN125678/0.18, Module 1.11.3 (Responses to CBER's IR #10 regarding Subgroup Analysis of ISS)

- STN125678/0.21, Module 1.11.3 (Responses to CBER's IR #13 regarding Cluster Distribution of Take)
- STN125678/0.27, Module 1.11.3 (Responses to CBER's IR # 8 and 16 regarding Pharmacovigilance Plan)
- STN125678/0.29, Module 1.11.3 (Responses to CBER's IR #21 regarding Take Rate)
- STN125678/0.34, Module 1.11.3 (Responses to CBER's IR #22 regarding Risk Management)
- STN125678/0.35, Module 1.11.3 (Responses to CBER's IR #24 regarding Discrepancies in Subgroup Subjects Numbers)
- STN125678/0.39, Module 1.11.3 (Responses to CBER's IR#26 regarding Troponin Data in Vaccinia Experienced Subset)
- STN125678/0.50, Module 1.11.3 (Responses to CBER's IR#32 regarding Re-calculation of PRNT Using CBER Accepted Imputation Approach

5.3 Table of Studies/Clinical Trials

The clinical trials that are considered essential to support the proposed indication and usage are reviewed in detail are summarized in Table 1.

Table 1: Overview of Clinical Trials that Are Essential to Support the Application

Study ID	**POX-MVA-005**	**POX-MVA-006**	**POX-MVA-008**	**POX-MVA-011**	**POX-MVA-013**	**POX-MVA-023**	**POX-MVA-024**
NCT ID	NCT00686582	NCT01913353	NCT00316602	NCT00316589	NCT01144637	NCT00686582	NCT00857493
Phase	2	3	2	2	3	2	2
IND Study	No	Yes	Yes	Yes	Yes	No	Yes
Study Sites	1 site in Germany	2 sites in South Korea	17 sites in the U.S. and 7 sites in Mexico	34 sites U.S and 2 in Puerto Rico	34 sites in U.S.	1 site in Germany	4 sites in U.S.
Study Design	Partially randomized, partially double-blind, placebo-controlled non-inferiority study to evaluate immunogenicity and safety of one and two doses of MVA-BN in healthy subjects	Two-site, open label, randomized, blinded immune analysis trial to compare vaccinia specific neutralizing antibody after two doses of MVA-BN with that after a single dose of ACAM2000, and to evaluate attenuation of ACAM2000 take reaction following prior vaccination with MVA-BN, in vaccinia naïve heathy military personnel	Multicenter, open-label, controlled study to evaluate safety and immunogenicity of MVA-BN in subjects with atopic dermatitis	Multicenter, open-label, controlled study to evaluate safety and immunogenicity of MVA-BN in vaccinia naïve and previously vaccinia-vaccinated HIV infected subjects	Randomized, double-blind, placebo-controlled lot consistency trial in vaccinia naïve healthy subjects	Open-label study to evaluate immunogenicity and safety of a single MVA-BN booster dose at two years after the MVA-BN primary vaccination in POX-MVA-005	Randomized, double-blind, placebo-controlled clinical trial to evaluate safety and immunogenicity of MVA-BN in elder populations
Subjects Planned	800	440	560 (230 healthy, 330 AD subjects)	550	4000	150	120
Subjects Enrolled	745	433	632 (282 healthy, 350 AD subjects	579 (97 Healthy, 482 HIV infected)	4005	152	120
Age Range (Years)	18-55	18-42	18-40	18-55	18-40	18-55	56-80

Study ID	POX-MVA-005	POX-MVA-006	POX-MVA-008	POX-MVA-011	POX-MVA-013	POX-MVA-023	POX-MVA-024
Treatment	A single dose of MVA-BN (1×10^8 Inf.U in 0.5 mL/dose) administered subcutaneously, or 2 doses at 28 days apart	**MVA Group:** Two doses of MVA-BN (1×10^8 Inf.U in 0.5 mL/dose) at 28 days apart followed by ACAM2000 vaccination at 28 days after the second dose of MVA-BN. **ACAM2000 Group:** a single dose of ACAM2000 ($2.5 - 12.5 \times 10^5$ plaque forming units of live vaccinia virus)	Two doses of MVA-BN (1×10^8 Inf.U in 0.5 mL/dose) at 28 days apart	A single dose of MVA-BN (1×10^8 Inf.U in 0.5 mL/dose) administered subcutaneously, or 2 doses at 28 days apart	MVA-BN 1×10^8 Inf.U in 0.5 mL/dose, 2 doses at 28 days apart	A single dose of MVA-BN (1×10^8 Inf.U in 0.5 mL/dose) administered subcutaneously	A single dose of MVA-BN (1×10^8 Inf.U in 0.5 mL/dose) administered subcutaneously, or 2 doses at 28 days apart
Primary Endpoints	Seroconversion rate determined by ELISA	Vaccinia specific neutralizing antibody determined by PRNT; Take attenuation	Vaccinia-specific ELISA seroconversion rate at Peak Visit	Serious adverse events and/or unexpected AEs	PRNT GMT at Peak Visit	Booster rate determined by ELISA (percentage of subjects with an ELISA titer ≥ cut-off value or any increase over baseline value for subjects with a pre-existing titer)	Serious adverse events (SAEs) associated with the study vaccine
Follow-up Duration	6 months after the last vaccination	6 months after the last vaccination	6 months after the last vaccination	6 months after the last vaccination	6 months after the last vaccination	6 months after the last vaccination	6 months after the last vaccination

5.4 Consultations

N/A

5.4.1 Advisory Committee Meeting

N/A

5.4.2 External Consults/Collaborations

N/A

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6. Discussion of Individual Studies/Clinical Trials

6.1 Trial #1

POX-MVA-006: A Randomized, Open-Label Phase 3 Non-Inferiority Trial to Compare Indicators of Efficacy for MVA-BN Smallpox Vaccine to ACAM2000 in 18-42 Year Old Healthy Vaccinia-Naïve Subjects

Reviewer's comment: *Contrary to the title, the study did not examine efficacy of the vaccine with a clinical endpoint but instead evaluated immunogenicity and take attenuation and no correlation of protection exists. Please also refer to Reviewer's comment on this in Section 6.1.8.2.*

6.1.1 Objectives

Co-Primary Objectives:

- To demonstrate non-inferiority of vaccinia specific neutralizing antibody responses at Peak Visit as determined by vaccinia-specific plaque reduction neutralization test (PRNT) between MVA-BN and ACAM2000

- To demonstrate the capacity of MVA-BN vaccination to attenuate take [in terms of maximum lesion area (MLA)] caused by subsequent scarification by ACAM2000

Secondary Objectives:

- To assess non-inferiority of MVA-BN compared to ACAM2000 in terms of vaccinia-specific enzyme-linked immunosorbent assay (ELISA) antibody response at the Peak Visits
- To assess seroconversion rates of MVA-BN compared to ACAM2000 at the Peak Visits
- To assess immune response dynamics in terms of antibody responses
- To assess the effect on the ACAM2000 vaccination take following MVA-BN priming
- To assess and compare safety and reactogenicity of vaccinations with MVA-BN and CAM2000 given alone or ACAM2000 given after MVA-BN priming

Reviewer's comments: *The Peak Visits were defined as the time points when the highest expected antibody titers would be observed. For MVA-BN, the Peak Visit was identified as two weeks after the last dose of the two-dose regimen administered 28 days apart. For ACAM2000, the Peak Visit was at four weeks after a single dose of ACAM2000.*

6.1.2 Design Overview

This study was a two-site, open-label, randomized, immune-analysis blinded Phase 3 trial to assess the effectiveness and safety of MVA-BN comparing to a US licensed smallpox vaccine ACAM2000 in approximately 440 smallpox vaccine naïve healthy US military personnel 18 through 42 years of age. The trials included two groups:

- Group 1: 220 vaccinia-naïve subjects receiving two 0.5 mL (1×10^8 Inf.U) doses of MVA-BN, SC four weeks apart followed by one dose of ACAM2000 ($2.5\text{-}12.5 \times 10^5$ plaque forming units) via scarification four weeks after the second MVA-BN vaccination.
- Group 2: 220 vaccinia-naïve subjects receiving one dose of ACAM2000 ($2.5\text{-}12.5 \times 10^5$ plaque forming units) via scarification, in accordance with USA military smallpox vaccine program.

The trial was designed to demonstrate effectiveness of MVA-BN by showing non-inferior vaccinia-neutralizing antibody responses, measured by PRNT at Peak Visit, compared to ACAM2000, and by showing the ability of prior MVA-BN vaccination to attenuate take induced by ACAM2000. Subjects were followed-up up to six months after the last vaccination (Refer to Section 6.1.7 for details).

Reviewer's comment: *The open-label study design to assess take attenuation has potential bias. Operational bias (e.g., ACAM2000 vaccination in Group 1)*

could not be excluded. Ideally, an adequate comparison would be take attenuation between subjects who previously received ACAM2000 and subjects who previously received MVA-BN. Please refer to Section 6.1.11 for details.

6.1.3 Population

Inclusion Criteria:

- Healthy male and female subjects, 18-42 years of age at date of informed consent signature, with acceptable baseline laboratory tests including troponin level <2 x upper limits of normal (ULN) and an ECG without clinically significant findings

Exclusion Criteria:

- Typical vaccinia scar
- Known or suspected history of smallpox vaccination defined as visible vaccination scar, documentation of smallpox vaccination, or subject report
- History of vaccination with any poxvirus-based vaccine
- History of any serious medical condition
- History of or active immunodeficiency or immunosuppression conditions
- History of or active autoimmune disease
- Uncontrolled serious infection, i.e., not responding to antimicrobial therapy
- History of malignancy, or clinical manifestation of severe hematological, renal, hepatic, pulmonary, central nervous, cardiovascular, or gastrointestinal disorders
- History of coronary heart disease, or any other heart conditions History of an immediate family member (father, mother, brother, or sister) who had onset of ischemic heart disease before the age of 50 years
- Clinically significant psychological disorder not adequately controlled by medical treatment
- History of anaphylaxis or any severe allergic reaction or serious adverse reaction to a vaccine

6.1.4 Study Treatments or Agents Mandated by the Protocol

Investigational Product: MVA-BN

MVA-BN was provided in LF aliquots with a titer range from 1 x 10^8 to 7.9 x 10^8 Infectious Units (Inf.U) of MVA-BN per milliliter (mL). The release titer was 2.5 x 10^8 to 7.9 x 10^8 Inf.U/mL. Therefore, a dose of 0.5 mL of MVA-BN LF formulation contained a nominal titer of 1 x 10^8 Inf.U.

Product lots and batches used in this study:

- Finished Drug Product: Lot Number: F00238

- Bulk Drug Substance: Batch Numbers (b) (4) with an expiry date of (b) (4) (The last vaccination date for MVA-BN was December 27, 2016).

Reviewer's comments: *In 2017, MVA-BN changed potency assay from the tissue culture infection based assay to a* (b) (4) *based assay. As a result, the reporting unit was changed from 50% tissue culture infectious dose ($TCID_{50}$) to Inf.U. The applicant states that the conversion factor between $TCID_{50}$ and Inf.U is 1:1, and the established limits and specifications for the product remain unchanged.*

For studies completed prior to 2017, the dose unit was reported as $TCID_{50}$ in the submission. Therefore, the dose unit of MVA-BN used in this review will be consistent with that reported in the corresponding individual studies.

Comparator Product: ACAM2000

As per the prescribing information, one dose of reconstituted ACAM2000 vaccine consisted of 2.5-12.5 x 10^5 plaque forming units of live vaccinia virus (VV).

Reviewer's comments: *In this study, ACAM2000 was supplied by the Military Vaccine Agency/Defense Health Agency. We requested that the applicant provide us with the accountability of ACAM2000 for our review. The applicant stated that they had no access to lot numbers, batch numbers or expiry dates and the Military Vaccine Agency/Defense Health Agency did not provide that information. Inability to account for the drug product proved to be a review issue that contributed to the take-attenuation endpoint not being included in the package insert. Please refer to section 6.1.11.2 for additional details.*

Study Treatments

Subjects in Group 1 received two 0.5 mL doses of MVA-BN administered SC at 28 days apart (Days 0 and 28) in the non-dominant upper arm.

Subjects in Group 1 also received a single dose of a droplet (0.0025 mL) of ACAM2000 administered by scarification using 15 jabs of a bifurcated needle four weeks after the second dose of MVA-BN (i.e., Day 56).

Subjects in Group 2 received a single dose of a droplet (0.0025 mL) of ACAM2000 vaccine (Day 0) administered by scarification using 15 jabs of a bifurcated needle.

Reviewer's comments: *MVA-BN was tested in three dose finding trials for safety and immunogenicity among healthy volunteers. Across these trials, a dose dependent response was observed for both ELISA and PRNT titers. The regimen of two-doses of MVA-BN, 1 x 10^8 Inf.U (or $TCID_{50}$) per dose,*

administered SC at 28 days apart was selected for the subsequent studies to support licensure. The dose finding studies were reviewed and documented in the Appendix of this review. Please refer to the Appendix for details.

6.1.5 Directions for Use

Please refer to Section 6.1.4 above.

6.1.6 Sites and Centers

The study was conducted in Brian Allgood Army Community Hospital located at Yongsan, South Korea APO AP 96205 (enrolled 199 subjects) and its Satellite site located at Dongducheon, South Korea APO AP 96205 (enrolled 241 subjects).

6.1.7 Surveillance/Monitoring

Study subjects were screened, evaluated, and monitored as scheduled in Table 2 (Group 1) and Table 3 (Group 2).

Table 2: Study Procedures-Group 1 (MVA-BN Arm)

Visit (V)	**Screen**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**Follow-up**
Week	-10~-1	0	1	2	4	5	6	8	9	10	12	34-38
Informed Consent	X											
Eligibility Criteria	X	X										
Randomization		X										
Medical History	X											
Physical Exam	X	X[1]	X[2]	X[1]	X[1]	X[2]	X[1]	X[1]	X[2]	X[1]	X[1]	X[2]
ECG	X			X			X[2]			X		
Troponin	X			X						X		
Vaccination		X			X			X[3]				
Handout of Memory aid		X			X			X				
Safety Lab	X			X			X			X		X[2]
Pregnancy Test	X	X			X			X			X	
Blood for Ab Analysis		X	X	X	X		X	X			X	
Photo of ACAM2000 Site									X	X		
AE/SAE/AESI		X	X	X	X	X	X	X	X	X	X	X

Source: Adapted from Table 4 of Module 5.3.5.1_POX-MVA-006 CSR (p46-48)
[1]Targeted physical exam
[2]Clinically indicated
[3]ACAM2000

Table 3: Study Procedures-Group 2 (ACAM2000 Arm)

Visit (V)	Screen	V1	V2	V3	V4	V5	V6	Follow-up
Week	-10~-1	0	1	2	4	6	8	26-30
Informed Consent	X							
Eligibility Criteria	X	X						
Randomization		X						
Medical History	X							
Physical Exam	X	X[1]	X[2]	X[1]	X[1]			X[2]
ECG	X			X			X[2]	
Troponin	X			X				
Vaccination		X						
Handout of Memory aid		X		X				
Safety Lab	X			X				X[2]
Pregnancy Test	X	X			X			
Blood for Ab Analysis		X	X	X	X		X	
Photo of ACAM2000 Site			X	X				
AE/SAE/AESI		X	X	X	X			X

Source: Adapted from Table 5 of Module 5.3.5.1_POX-MVA-006 CSR (p49-51)
[1]Targeted physical exam
[2]Clinically indicated

6.1.7.1 Safety Monitoring

Following vaccination subjects were kept under close observation for at least 30 minutes, with appropriate medical treatment readily available in case of a rare anaphylactic reaction following the administration of the vaccine.

After each vaccination subjects received a memory aid, a ruler and thermometer to record solicited injection-site and systemic adverse reactions and its corresponding intensity for 15 days after each vaccination (days 0-14). Solicited injection-site reactions included injection-site pain, erythema, swelling, induration, and pruritus. Subjects were also asked to record injection-site appearance (normal/healed, red spot, bump, reddish blister, whitish blister, scab, ulcer/crater, warmth, swollen >3 inches, red streaks, drainage). Solicited systemic reactions included body temperature, headache, myalgia, chills, nausea, fatigue, malaise and swollen lymph nodes.

Safety laboratory values were measured as scheduled in the study procedures and performed at the site specific local laboratory.

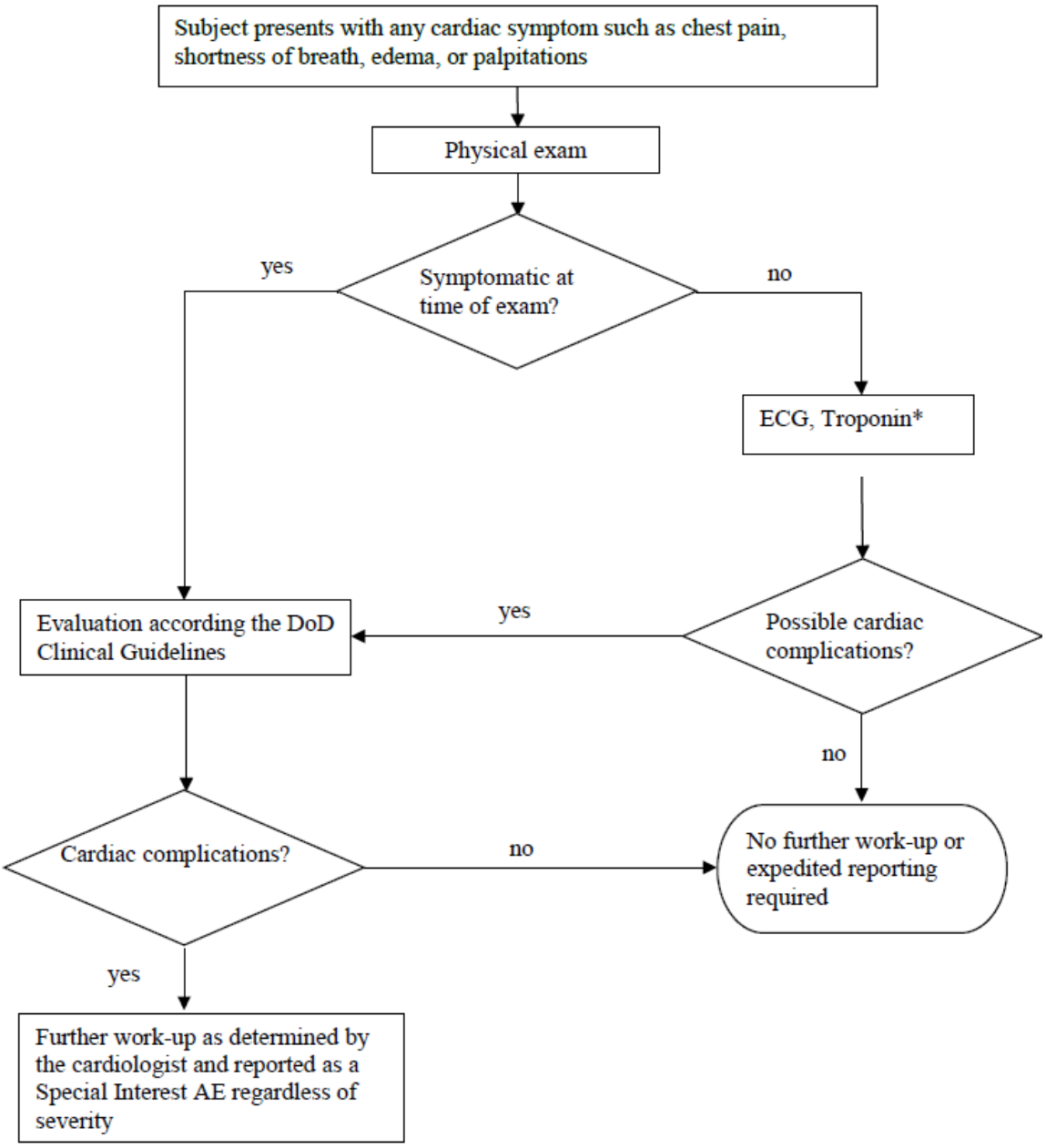
Unsolicited adverse events (AEs) were monitored for 28 days (with a window of 28-35 days) after each vaccination. SAEs and AESIs were monitored from the first study vaccination through at least six months after ACAM2000 scarification for both Group 1 and Group 2.

Cardiac events consistent with cases of myo-/pericarditis were collected as AESIs. Myo-pericarditis was defined as:

- Any cardiac sign or symptom (e.g., chest pain, shortness of breath etc.) developed since the first vaccination
- ECG abnormalities determined to be clinically significant by the investigating physician
- Cardiac enzyme Troponin I ≥ 2 x ULN

Potential cases of myo-pericarditis were assessed as described in the algorithm below (Fig 1).

Figure 1: Algorithm for Assessment of Cardiac Events



*At any protocol-scheduled ECG and/or troponin I abnormality, the algorithm will begin at this point.

The trial had a Data and Safety Monitoring Board (DSMB) to oversee the safety of study subjects. The DSMB consisted of five independent members with expertise in infectious disease, cardiology and epidemiology as well as a biostatistician. The DSMB reviewed safety data and monitored the trial progress periodically.

6.1.7.2 Indicators of Efficacy Monitoring

Immunogenicity Monitoring

Blood was drawn for antibody analysis as scheduled in Tables 2 and 3. The immune responses were measured by blinded operators using vaccinia virus Western Reserve (VV-WR) strain based PRNT and MVA-BN based ELISA.

Reviewer's comments: *The PRNT and ELISA analytic methods were established and validated at BN and the tests were performed at BN's facility in* (b) (4) *. The standard operation procedure for the ELISA (SOP BN0002809) and the PRNT (SOP BN0003536) were submitted under Module 5.3.1.4.*

Skin Lesion Monitoring

A digital photograph was taken of the vaccination site using the (b) (4) camera system at 6-8 and 13-15 days after scarification with ACAM2000. The size (i.e., area and diameter) of skin lesions were assessed by a blinded Independent Take Review Committee (ITRC). The ITRC consisted of three expert physicians in take assessment.

The ITRC chart provided detailed guidance regarding the take definition and assessment. In summary, take was categorized as full take (visible lesion with a maximal diameter ≥ 5mm), partial take (visible lesion with a maximal diameter < 5mm), and absent take (no visible lesion) measured at days 6-8. Two primary assessors of the ITRC independently made the initial assessment of all the takes. After the two primary ITRC assessors made the initial assessment of all the takes, if differences of opinion existed, then the respective case was referred by the biostatistician to the third ITRC member for independent assessment. If there was still ambiguity, then the three ITRC members would convene to discuss If there was a 2:1 split-decision, then the majority decision would be final and the discrepancy would be recorded on the ITRC Take Assessment Form. In the event of a split 1:1:1 decision after the ITRC has consulted together, then the assessment would be classified as "no-consensus."

The maximal lesion diameter (MLD) was defined as the largest diameter measured across the lesion on Day 6-8, or Day 13-15, after ACAM2000 scarification. The maximal lesion area (MLA) was defined as the maximum of two measurements on Day 6-8 or Day 13-15. If one of the lesion areas was missing, then the MLA was calculated as the single lesion area. However, subjects with

only one lesion area recorded were considered a major protocol violation and were excluded from the per protocol set (PPS).

6.1.8 Endpoints and Criteria for Study Success

6.1.8.1 Co-Primary Endpoints

- PRNT geometric mean titer (GMT) at the Peak Visits
- MLA in mm^2 after scarification with ACAM2000

6.1.8.2 Secondary Endpoints

Immunogenicity Endpoints

- GMTs at the individual peak measured by vaccinia-specific PRNT
- GMTs at all antibody blood sample time points measured by vaccinia-specific PRNT
- PRNT seroconversion rates at Peak Visits defined as the percentage of initially seronegative subjects with appearance of antibody titers equal or greater than the assay cut-off value (ACV) in a vaccinia-specific PRNT

The non-inferiority margin for the secondary endpoints of PRNT GMTs was the same as the co-primary immunogenicity endpoint, 0.301 on the log10 titer.

Reviewer's comments: *The CSR indicates that "the assay cut-off value for the vaccinia-specific ELISA can be found in the SOP BN0002809 and for the vaccinia specific PRNT can be found in the SOP BN0003536." However, we were unable to find the ACV except for a cut-off value for ELISA OD (0.35). We did see in the CSR for POX-MVA-005 that the ACVs for ELISA and PRNT were 1:50 and 1:6, respectively. An information request (IR) regarding the ACVs for each study and each assay was sent to the applicant on December 21, 2018. The applicant stated in its response (STN125678/0.9) that the PRNT ACVs for POX-MVA-006, -013 and -037 were 2; for POX-MVA-005, -011 and -023 were 6; and for POX-MVA-008, and -024 were 15; and the ELISA ACV for all trials was 50.*

Please note that various versions of PRNT were used across the studies, and the LLOQ varied from 20 (b) (4)*. In the similar way, the LLOD varied from* (b) (4)*. Therefore, PRNT GMT cannot be compared across studies. As described in Section 4.2, only PRNT Version* (b) (4) *(used in POX-MVA-013) and Version* (b) (4) *(used in POX-MVA-006) were validated and accepted by CBER assay reviewers.*

Take Attenuation Endpoints

- Investigator-measured MLD in mm after scarification with ACAM2000
- Investigator-measured lesion diameter in mm at Days 6-8 and 13-15 after scarification with ACAM2000
- The individual take was classified as either full, partial, or absent take by a blinded ITRC

Reviewer's comments: *In the protocol and study report for POX-MVA-006 the applicant called the take attenuation endpoints "efficacy endpoints." While we agreed that take attenuation represents control of injection site vaccine virus replication conferred by the immune response to previous vaccination and agreed to include it as a co-primary endpoint, it has not been established that an attenuation or a certain degree of attenuation on its own is predictive of protection against smallpox. In this review, the endpoints will be called take attenuation endpoints.*

Safety and Reactogenicity Endpoints

- Occurrence, relationship to vaccine, and intensity of any SAE
- Occurrence of any cardiac sign or symptom indicating a case of myo-/pericarditis
- Occurrence of any Grade 3 or 4 AEs possibly, probably, or definitely related to the vaccine within 28 days after each vaccination
- Occurrence, relationship to vaccine, and intensity of any non-serious AEs within 28 days after each vaccination
- Occurrence of solicited systemic reactions within 15 days after each vaccination (Days 0-14): intensity, duration, and relationship to vaccination
- Occurrence of solicited local reactions within 15 days after each vaccination (Days 0-14): intensity and duration
- Daily measurement of major lesion size, major erythema, and major induration diameter (mm) based on physical appearance of vaccination site as documented in the memory aid

6.1.9 Statistical Considerations & Statistical Analysis Plan

6.1.9.1 Study Hypotheses and Analyses of Primary Endpoints

The trial had two co-hypotheses: immunogenicity hypothesis and take attenuation hypothesis.

Immunogenicity Hypothesis and Analyses

The immunogenicity co-primary endpoint was to assess non-inferiority of MVA-BN compared to ACAM2000 in terms of antibody response GMT at Peak Visits determined by PRNT.

Suppose m1 was the PRNT GMT in Group 1 (two weeks after the second dose of MVA-BN) and m2 was the PRNT GMT in Group 2 (four weeks after a single dose of ACAM2000). The null hypothesis (H0) and the alternative hypothesis (H1) were:

$$H0: m1 - m2 \leq -\Delta$$

versus

$$H1: m1 - m2 > -\Delta$$

Where Δ was the non-inferiority margin. For the PRNT the non-inferiority margin was pre-specified at 0.301 on the log10 scale (equivalent to a doubling on the original titer scale for the GMT).

The above hypotheses were tested using a *t*-test on the difference between the two log transformed peak PRNT GMT values based on the assumption that the log10 titer values had a normal distribution.

If the LB was above –Δ then the null hypothesis would be rejected and the non-inferiority of MVA-BN to ACAM2000 would be demonstrated.

Reviewer's comment: *The margin Δ for the non-inferiority PRNT GMT comparison was the same as that used for the assessment of non-inferiority of ACAM2000 vs. Dryvax in the BLA application of ACAM2000.*

Take Attenuation Hypothesis

The take attenuation co-primary hypothesis of the trial was to assess if the median of the MLA following ACAM2000 vaccination was significantly reduced for subjects in Group 1 who received prior MVA-BN vaccinations compared to those in Group 2 who received no prior MVA-BN vaccinations. Area of attenuation rate (AAR) was calculated as 1 minus the ratio of median MLA in Group 1 (M1) over the median MLA in Group 2 (M2), i.e., 1-M1/M2.

The null hypothesis H0 was AAR less than or equal to a pre-defined constant λ, H0: AAR≤ λ, which was tested against the one-sided alternative hypothesis (H1) that the ratio > λ, H1: AAR> λ.

The value of λ was defined to be 40% which was based on the study reported by Talbot TR et al[8] that the area of vaccination lesion site was reduced between 37.7% to 41.5% following vaccination with a frozen Aventis Pasteur Smallpox Vaccine (also known as Wetvax), in subjects previously vaccinated with Dryvax compared to vaccinia-naïve subjects.

The test was performed using Hodges-Lehmann method. Specifically, only the upper 95% CI of the Hodges-Lehmann estimator was used with a one-sided significance level of 2.5% that needed to be below (1-λ) =0.6 for the null hypothesis to be rejected, and the co-primary endpoint to be met. Otherwise, the null hypothesis would not be rejected and the co-primary endpoint would not be met.

6.1.9.2 Sample Size Calculation

The immunogenicity co-primary endpoint was used for sample size calculation. Based on previous studies with MVA-BN, the standard deviation (SD) of log10 mean titers at two weeks following two doses of MVA-BN was approximately 0.866. Assuming a significance level of 5%, the sample size for each group to

reach a power of ≥ 90% was calculated as 175 subjects. In order to account for an approximately 20% rate for exclusion from the PPS, a total of 220 subjects were required for each of the two groups.

Using this data, a simple case resampling non-parametric bootstrap simulation was performed to estimate the likely ratio of the medians of the maximum lesion area that could be detected using the above test with 90% power. The proposed sample size of n=175 subjects in the PPS of each group gave a power at least 90% to show a significant reduction in the median of Group 1 compared to the median of Group 2 of at least λ=40%.

6.1.9.3 Methods of Handling Missing Data

Analysis of immunogenicity variables were done on a valid case basis, i.e., for missing observations no imputation technique such as “Last observation carried forward” (LOCF) were applied, since this could introduce an optimistic bias into the analysis.

For the analysis of safety data, incomplete AE and medication start and end dates were imputed in order to assign these events to the vaccination period as described below:

For Group 1:

- Vaccination Period 1 covered from Visit 1 (immediately after the first vaccination) until Visit 4 (just before the second vaccination)
- Vaccination Period 2 covered from Visit 4 (immediately after the second vaccination) until Visit 7 (just before the third vaccination)
- Vaccination Period 3 covered from Visit 7 (immediately after the third vaccination) until Visit 10
- The Overall MVA-BN Vaccination Period was the combination of Vaccination Periods 1 and 2

For Group 2:

- Vaccination Period 1 covered from Visit 1 (immediately after the first vaccination) until Visit 4

If start time was missing and start date of AE coincided with the date of a vaccination, the AE was assigned to the vaccination period corresponding to the vaccination on this date.

6.1.9.4 Interim Analysis

No interim analysis was planned for this trial.

6.1.9.5 Safety Analyses

<u>Serious Adverse Events (SAE)</u>

The number and percentage of subjects experiencing an SAE as well as the number of events are summarized by period. The number of subjects with at least one SAE was compared between the groups by means of Fisher's exact test.

Adverse Events of Special Interest (AESI)

AESIs were reported separately but in the same way as SAEs.

Solicited AEs

Solicited injection-site and systemic AEs were summarized by preferred term (PT) after each vaccination and broken down by maximum intensity.

Unsolicited AEs

Treatment-emergent unsolicited AEs were summarized by system organ class (SOC) and PT. A summary of the number of AEs by intensity and by relationship to trial vaccination was also presented.

The number of AEs with a reasonable possibility of being related to the vaccine was presented in a separate listing and summarized in a table by SOC and PT and vaccination period and by subject.

MedDRA (Medical Dictionary for Regulatory Activities) version 20.0 was used for coding AEs.

6.1.10 Study Population and Disposition

6.1.10.1 Populations Enrolled/Analyzed

Full Analysis Set (FAS)

FAS was defined as all subjects who had received at least one dose of trial vaccine and for whom any post vaccination safety or immunogenicity data were available.

The safety analysis and secondary supportive immunogenicity analyses were performed on the FAS.

Per Protocol Set for Immunogenicity (PPS-IMM)

The PPS-IMM was defined as the subset of subjects in the FAS who received all vaccinations, completed all visits up until Visit 7 (four weeks after the second dose of MVA-BN) for Group 1 and Visit 4 (four weeks after a single dose of

ACMA2000) for Group 2, and adhered to all protocol conditions pertaining to immunogenicity without major protocol violation.

Major protocol violation included:

1. Premature discontinuation of the trial
2. Subject did not meet all the inclusion criteria
3. Subject met one or more of the exclusion criteria
4. Withdrawal from the second vaccination (Group 1)
5. Major vaccine preparation and administration deviation from specification as given in the protocol including cases where the subject fulfilled at least one of the criteria specified in the protocol for withdrawal from vaccination
6. Major deviations of the visit window *pertaining to collection of immunogenicity data* as determined during the data review committee (DRM)
7. Unallowed prior or concomitant medication
8. Missing ELISA or PRNT titers at trial Day 0 or Day 42 for subjects in Group 1 and at trial Day 0 or Day 28 post ACAM2000 vaccination Group 2.

The analysis of the co-primary endpoint for immunogenicity was based on the PPS-IMM.

PPS for Take Attenuation (PPS-Take)

The PPS-Take was defined as the subset of subjects in the FAS who had received all vaccinations, completed all visits up until four weeks after ACAM2000 scarification (Visit 10 for Group 1, and Visit 4 for Group 2), and adhered to all protocol conditions without major protocol violation.

Major protocol violation included:

1. Premature discontinuation of the trial
2. Subject did not meet all of the inclusion criteria
3. Subject met one or more of the exclusion criteria
4. Withdrawal from the second or third vaccination (Group 1)
5. Major vaccine preparation and administration deviation from specification as given in the protocol including cases where the subject fulfilled at least one of the criteria specified in the protocol for withdrawal from vaccination
6. Major deviations of the visit window as determined during the data review window
7. Unallowed prior or concomitant medication
8. Missing lesion area data on Day 6-8 or Day 13-15 after ACAM2000 vaccination
9. Missing ELISA or PRNT titers at trial Day 0, Day 42 for subjects in Group 1 or Day 28 post ACAM2000 vaccination (Groups 1 and 2).

The analysis of the co-primary endpoint of take attenuation was based on the PPS-Take.

6.1.10.1.1 Demographics

Study subjects were recruited from current Department of Defense (DoD) personnel. The demographic and baseline characteristics are summarized in Table 4. Overall, the mean subject age was 23.5 years (range: 18-42 years), with most subjects in the 18-24 year age range (69.5% in each group). A greater proportion of the subjects was male [365 subjects (84.3%)], White/Caucasian [262 subjects (60.5%)], and Non-Hispanic or Latino [339 subjects (78.3%)]. Age, ethnicity, race and gender were similar between the two groups.

Table 4: Baseline Demographic and Characteristics

Characteristics	**Group 1 (MVA-BN) N=220**	**Group 2 (ACAM2000) N=213**	**Total N=433**
Age (Years)			
Mean (SD)	23.5 (4.77)	23.4 (4.58)	23.5 (4.67)
Median	22.0	22.0	22.0
Range	18-42	18-41	18-42
Age Band, n (%)			
18-24 Years	153 (69.5)	148 (69.5)	301 (69.5)
25-34 Years	58 (26.4)	56 (26.3)	114 (26.3)
35-42 Years	9 (4.1)	9 (4.2)	18 (4.2)
Sex, n (%)			
Female	39 (17.7)	29 (13.6)	68 (15.7)
Male	181 (82.3)	184 (86.4)	365 (84.3)
Race, n (%)			
American Indian or Alaskan Native	8 (3.6)	6 (2.8)	14 (3.2)
Asian	14 (6.4)	12 (5.6)	26 (6.0)
Black	48 (21.8)	40 (18.8)	88 (20.3)
Native Hawaiian/Pacific Islander	5 (2.3)	3 (1.4)	8 (1.8)
White	126 (57.3)	136 (63.8)	262 (61.5)
Other	19 (8.6)	16 (7.5)	35 (8.1)
Ethnicity, n (%)			
Hispanic or Latino	54 (24.5)	40 (18.8)	94 (21.7)
Non-Hispanic/Latino	166 (75.5)	173 (81.2)	339 (78.3)

Source: Adapted from Table 9 of POX-MVA-006 CSR (page 98-99) under Module 5.3.5.1, STN125678/0.
N=total number of subjects in the group, n=number of subjects in the corresponding category.

6.1.10.1.2 Medical/Behavioral Characterization of the Enrolled Population

Each subject's cardiac risk factor was calculated before the first vaccination, and any subject with a 10% or greater risk of developing a myocardial infarction or coronary death within the next 10 years were excluded from trial participation. The cardiac risk factor was well balanced between the two groups. Specifically, the mean cardiac risk factor (± SD) for Group 1 and Group 2 was 1.2% ± 0.56%

and 1.1% ± 0.39%, respectively. In addition, 46 subjects (20.9%) in Group 1 and 40 subjects (19.9%) in Group were categorized as smokers.

6.1.10.1.3 Subject Disposition

Subject disposition is presented in Table 5. Overall, 440 subjects were randomized, 221 subjects in Group 1 and 219 subjects in Group 2.

In Group 1, 220 subjects (99.5%) received the first MVA-BN vaccination, 208 subjects (94.1%) received the second MVA-BN vaccination, and 196 subjects (88.7%) received the ACAM2000 vaccination. No subjects withdrew from the second MVA-BN vaccination or the ACAM2000 vaccination. In Group 2, 213 subjects (97.3%) received the ACAM2000 vaccination.

Seven subjects were randomized but not treated, one in Group 1 (Subject (b) (6)) and six in Group 2. Reasons for not receiving vaccination for these subjects are listed below:

- Subject (b) (6) : medical response technician deemed subject not to be a candidate upon re-evaluation at Visit 1
- Subject 1(b) (6) : acne at baseline that was slightly improved but still pustular at Visit 1
- Subject (b) (6) and Subject (b) (6) : consent withdrawal after randomization
- Subject (b) (6) : development of a disqualifying rash
- Subject (b) (6) : not complying with the visit schedule due to work responsibilities
- Subject (b) (6) : a new diagnosis after inclusion/exclusion criteria was reviewed

Reviewer's comment: *The specific reason why Subject* (b) (6) *was deemed not eligible for treatment was not described in the report. The new diagnosis for Subject* (b) (6) *was not specified.*

Table 5: Study Subject Disposition-Study POX-MVA-006

Disposition	Group 1 N (%)	Group 2 N (%)	Total N (%)
Subjects screened			637
Subjects randomized	221	219	440
Subjects received at least one injection	220 (99.5)	213 (97.3)	433 (98.4)
Subjects received two injections	208 (94.1)	NA	NA
Subjects received three injections	196 (88.7)	NA	NA
Discontinued from study	32 (14.5)	15 (6.8)	47 (10.7)
Adverse event	2 (0.9)	0 (0.0)	2 (0.5)
Subject's request	7 (3.2)	2 (0.9)	9 (2.0)
Subject non-compliance	13 (5.9)	4 (1.8)	17 (3.9)
Physician Request	0 (0.0)	2 (0.9)	2 (0.5)
Other	10 (4.5)	7 (3.2)	17 (3.9)

Source: Adapted from Figure 1 of POX-MVA-006 CSR (page 95), STN125678/0, Module 5.3.5.1.
NA: Not applicable.
N: number of subjects.

Reviewer's comment: *More than twice as many subjects discontinued from study Group 1(n=32, 14.5%) than from study Group 2 (n=15, 6.8%). The major reasons for discontinuation were refusal to receive ACAM2000 or inability to comply with scheduled study visits (due to training, returning to the USA). Only two subjects discontinued study due to an adverse event, both in Group 1. The two AEs were chest pain and tibia fracture and were considered not related to MVA-BN vaccination. Please refer to Sections 6.1.12.4 and 6.1.12.5 for details.*

The higher percentage of subject discontinuation in Group 1 was likely because Group 1 subjects were required to receive two more injections compared with Group 2. Therefore, the apparently unbalance in subject disposition between the two groups would unlikely affect overall conclusions.

6.1.11 Efficacy Analyses

6.1.11.1 Analyses of Primary Endpoint(s)

Analyses of Co-Primary Endpoint: Immunogenicity

The co-primary immunogenicity endpoint of vaccinia-specific neutralizing antibody as determined by VV-WR based PRNT at peak visits was evaluated on the PPS-IMM population. The results are presented in Table 6. The LB of Group 1/Group 2 GMT ratio at the Peak Visit was above the pre-specified margin of 0.5. Therefore, non-inferiority of MVA-BN compared with ACAM2000 in anti-vaccinia neutralization antibody titer was met.

Reviewer's comment: *The PRNT assay used in this trial was re-validated and accepted by CBER assay reviewers. The LLOD and LLOQ for the PRNT was set to* (b)(4) *and 20, respectively. The data presented in Tables 6 and 9 reflect the changes.*

Table 6: Non-Inferiority Comparison of Anti-Vaccinia Antibody Geometric Mean Titers (GMT) Determined by Plaque Reduction Neutralization Test (PRNT)-PPS-IMM Population

Time Point	Group 1 (N=185) GMT (95% CI)	Group 2 (N=186) GMT (95% CI)	Group 1/Group 2 GMT Ratio (97.5% CI)
Baseline	10.1 (9.9, 10.2)	10.0 (10.0, 10.0)	1.0 (0.99, 1.02)
Peak Visit	152.8 (133.3, 175.0)	84.4 (73.4, 97.0)	1.8 (1.49, 2.20)

Source: Adapted from Table 1 and Table 2 of POX-MVA-006 CSR (page 5), STN125678/0.50, Module 1.11.3, Responses to IR32
PRNT GMT values below LLOQ were imputed as 1/2 LLOQ.

Reviewer's comments: *Analysis of the PRNT GMTs at peak visits was also conducted in the FAS population. The PRNT GMTs were 153.0 (95%CI: 134.3, 174.3) and 76.6 (95%CI: 64.9, 90.4), for Group 1 (N=220) and Group 2 (N=213), respectively, which were similar to those in the PPS-IMM population.*

Analyses of Co-Primary Endpoint: MLA

The median skin lesion areas at Day 6-8 and Day 13-15 after ACAM2000 vaccination and the median MLA in Groups 1 and 2 are presented in Table 7. A significant reduction in skin lesion areas in MVA-BN immunized subjects (Group 1) was observed compared with subjects who did not receive MVA-BN (Group 2). The AAR for MLA in MVA-BN immunized subjects was 97.9% with a 95% CI LB o of 96.6%, which was greater than the protocol specified margin 40% (Table 7). Therefore, the success criterion for this co-primary endpoint was met.

The skin lesion attenuation measured at Day 6-8 and Day 13-15 was similar to that of the MLA (Table 7).

Table 7: Comparison of Skin Lesion Areas After ACAM2000 Vaccination-PPS-Take

	Group 1 (N=165) Median (95%CI)	Group 2 (N=161) Median (95%CI)	AAR % (95%CI)
Day 6-8	0.0 (0.0, 1.0)	37.0 (33.0, 42.0)	95.2 (93.8, 96.2)
Day 13-15	0.0 (0.0, 0.0)	75.0 (69.0, 85.0)	98.2 (97.7, 98.4)
Maximum	0.0 (0.0, 2.0)	76.0 (70.0, 87.0)	97.9 (96.6, 98.3)

Source: Adapted from Table 12 of POX-MVA-006 CSR (page 103), STN125678-0, Module 5.3.5.1.
Note: Median=median of skin lesion area expressed as mm^2; Area attenuation ratio (AAR)=1-(Median in Group 1)/(Median in Group 2); N=number of subjects in the corresponding PPS-Take population.

Reviewer's comments: *Skin lesion areas at Day 6-8 and Day 13-15 after ACAM2000 vaccination were two of the protocol specified secondary endpoints. The data are presented in this section for conciseness.*

The AAR for MLA in MVA-BN vaccinated subjects assessed using the FAS population (N=196 for Group 1 and N=213 for Group 2) was 97.9% (96.8%, 98.3%), which was the same as that in PPS-Take population.

Reviewer's comments: *Most of subjects who were excluded from PPS-Take were due to major protocol violations, specifically a skin lesion photo taken more than one day outside the protocol defined visit windows (i.e., 6-8 days and 13-15 days after vaccination with ACAM2000) or not having both photos at 6-8 days and 13-15 days after ACAM2000 vaccination. In total, 38 subjects (28 subjects with missing photos and 10 subjects with violations of the visit windows) in Group 1 and 33 subjects (6 subjects with missing photos and 27 with violations of the visit windows) in Group 2 violated the criteria. The percentage of subjects with major protocol violations appears high, 19.4% or 38 out of 196 ACAM2000 vaccinated subjects in Group 1, and 15.5% or 33 out of 213 ACAM2000 vaccinated subjects in Group 2.*

The degree of take attenuation was more dramatic compared to the results reported in POX-MVA-002, in which skin lesion area was reduced about 18-38% (please refer to the appendix of this review for details). Please refer to Reviewer's the comment in Section 6.1.11.2 for further discussion.

6.1.11.2 Analyses of Secondary Endpoints

Analyses of Secondary Immunogenicity Endpoints

Comparison of PRNT GMTs at Various Time Points after Vaccination

Summary of the vaccinia specific PRNT GMTs at various time points after vaccination with either MVA-BN (Group 1) or ACAM2000 (Group 2) for the PPS-IMM population are presented in Table 8. One dose of MVA-BN vaccination elicited only modest vaccinia specific antibody response as measured at two and four weeks after vaccination, and the specific antibody response increased significantly after the second dose of MVA-BN and peaked at two weeks after the second dose (Table 8). In contrast, a single dose of ACAM2000 vaccination induced significant antibody response which peaked at four weeks after the vaccination (Table 8).

Table 8: Vaccinia Specific Neutralizing Antibody Titers Determined by Plaque Reduction Neutralization Test (PRNT) at Various Time Points after Vaccination-PPS-IMM

Time Point	Group 1 (N=185) GMT (95% CI) [n]	Group 2 (N=186) GMT (95% CI) [n]
Baseline	10.1 (9.9, 10.2) [185]	10.0 (10.0, 10.0) [186]
Wks after the 1st Vaccination		
Two	23.4 (20.5, 26.7) [184]	23.7 (20.9, 26.8) [184]
Four	23.5 (20.6, 26.9) [185]	84.4 (73.4, 97.0) [186]
Eight	NA	72.3 (63.7, 82.1) [183]
Wks after the 2nd Vaccination		
Two	152.8 (133.3, 175.0) [185]	NA
Four	118.6 (103.5, 135.9) [179]	NA
Eight	100.5 (84.9, 118.9) [172]	NA

Source: Adapted from Table 1 of POX-MVA-006 CSR (page 5), STN125678/0.50, Module 1.11.3, Responses to IR32.
Notes: N=number of subjects in the specific group; n=number of subjects with data available; GMT=geometric mean titer; NA=not applicable; Wks=weeks.
PRNT GMT values below LLOQ were imputed as1/2 LLOQ.

Seroconversion Rate Based on PRNT Titer at Peak Visit

Seroconversion rates determined by PRNT at baseline and Peak Visit for Group 1 and Group 2 are provided in Table 9.

Table 9: Comparison of Seroconversion Rate Determined by PRNT at Peak Visit-PPS-IMM

Time Point	Group 1 (N=185) SC % (95% CI) [n/m]	Group 2 (N=186) SC % (95% CI) [n/m]
SCR at Baseline	8.7 (5.1, 13.8) [16/183]	1.6 (0.3, 4.7) [3/184]
SCR At Peak Visit	100.0 (98.0, 100.0) [185/185]	97.3 (93.8, 99.1) [181/186]

Source: Adapted from Table 17 (page 110) and Table 20 (page 115) of POX-MVA-006 CSR, STN125678/0, Module 5.3.5.1.
Notes: SCR=seroconversion rate; N=total number of subjects in the specific group; n=number of subjects with seroconversion; m=number of subjects with data available. Seroconversion was defined as a PRNT titer of >2.

Analyses of Secondary Take Endpoints

Comparison of Investigator-Measured Lesion Diameter

The Investigator-measured MLD, Day 6-8 diameter, and Day 13-15 diameter are presented in Table 10.

The median MLD in Groups 1 and 2 was 0.0 mm (95% CI: 0.0, 2.0) and 11.0 mm (95% CI: 10.0, 11.0), respectively. The median MLD for subjects that received

two doses of MVA-BN prior to ACAM2000 scarification was reduced 87.5% (95% CI: 83.3, 88.9) compared to those who did not (Table 10).

Similarly, the median lesion diameters at Day 6-8 and Day 13-15 after ACAM2000 scarification were also statistically significantly reduced in subjects in Group 1 as compared with Group 2. The diameter attenuation ratios (DAR) at Day 6-8 and Day 13-15 were 80.0% (95% CI: 77.8, 85.7) and 88.9% (95% CI: 87.5, 90.0), respectively (Table 10).

Table 10: Statistical Analyses of Investigator-Measured Lesion Diameter After ACAM2000 Scarification-PPS-Take

	Group 1 (N=165) Median (95%CI)	**Group 2 (N=161) Median (95%CI)**	**DAR % (95%CI)**
Day 6-8	0.0 (0.0, 2.0)	8.0 (8.0, 9.0)	80.0 (77.8, 85.7)
Day 13-15	0.0 (0.0, 0.0)	10.0 (10.0, 11.0)	88.9 (87.5, 90.0)
Maximum	0.0 (0.0, 2.0)	11.0 (10.0, 11.0)	87.5 (83.3, 88.9)

Source: Adapted from Table 13 of POX-MVA-006 CSR (page 104), STN125678-0, Module 5.3.5.1.
Note: Median=median diameter of skin lesion expressed as mm; DAR=1-(Median in Group 1)/(Median in Group 2); N=number of subjects in the corresponding PPS-Take population.

Analyses of Take after ACAM2000 Scarification in Subjects Previously Vaccinated with MVA-BN

Each individual take was classified as either full, partial, or absent by the ITRC. The proportions of subjects in PPS-Take population with (full or partial) or without a take after ACAM2000 scarification are presented in Table 11. The majority (53.9%) of the subjects who received two doses of MVA-BN prior to ACAM2000 vaccination had no takes, while the remainder (45.1%) had partial or full takes (23.0% each) (Table 11).

Table 11: Statistical Analyses of Take Classification after ACAM2000 Scarification-PPS-Take

Type of Take	**Group 1 (MVA-BN) % (95% CI) [n/N]**	**Group 2 (ACAM2000) % (95% CI) [n/N]**	**Group 1-Group 2 % (95% CI)**
Full Take	23.0 (16.8, 302) [38/165]	92.5 (87.3, 96.1) [149/161]	-69.5 (-76.8, -61.2)
Partial Take	23.0 (16.8, 302) [38/165]	4.3 (1.8, 8.8) [7/161]	18.7 (7.9, 29.2)
Absent Take	53.9 (46.0, 61.7) [89/165]	1.9 (0.4, 5.3) [3/161]	52.1 (42.2, 61.0)

Source: Adapted from Table 14 (page 105) of POX-MVA-006 CSR, STN125678/0, Module 5.3.5.1.
Notes: N=number of subjects in the specific group; n=number of subjects with available data for the indicated event; two subjects in Group 2 had no data regarding take status.

Similar results were also obtained in the FAS population. Specifically, the proportions of subjects in Group 1 with a full, partial and absent take were 20% (95% CI: 14.9, 25.9), 19.5% (95% CI: 14.5, 25.4) and 49.1% (95% CI: 42.3, 55.9), respectively. The proportions of subjects in Group 2 with a full, partial and absent take were 90.1% (95% CI: 85.3, 93.8), 4.2% (95% CI: 2.0, 7.9) and 2.8% (95% CI: 1.0, 6.0), respectively.

To explore the potential explanations for the unexpected low take rate in Group 1 subjects, PRNT GMTs prior to ACAM2000 vaccination and four weeks after ACAM2000 vaccination were analyzed among Group 1 subjects stratified by take type. As shown in Table 12, PRNT GMTs prior to ACAM2000 were similar between the subjects who had any take (PRNT GMT=117.2) and the subjects who did not have a take (PRNT GMT=122.9) following ACAM2000 vaccination. However, four weeks after ACAM2000 vaccination, PRNT GMT among subjects who did not have a take was significantly lower than the pre-ACAM2000 vaccination GMT (55.3 vs. 122.9), while the PRNT GMT among subjects who had any take indicated a significant booster of antibody response compared to the pre-ACAM2000 vaccination PRNT GMT (198.3 vs. 117.2). A similar observation was also demonstrated by antibody responses measured by ELISA (Table 12).

Table 12: Comparison of Vaccinia Specific Antibody Titers Prior to and Post ACAM2000 Vaccination Among Group 1 Subjects Stratified by Take Type (PPS-Take)

Subjects and Time Point	N	PRNT GMT (95% CI)	ELISA GMT (95% CI)
All Group 1 Subjects			
Prior to ACAM2000 (Week 8)	165	120.2 (104.0, 139.0)	676.3 (596.3, 766.9)
Post ACAM2000 (Week 12)	162	99.9 (82.6, 120.9)	556.0 (483.8, 639.0)
Subjects with a Full Take			
Prior to ACAM2000 (Week 8)	38	112.5 (85.5, 148.0)	541.8 (414.6, 707.8)
Post ACAM2000 (Week 12)	38	276.6 (193.2, 396.2)	1096.6 (869.3, 1383.3)
Subjects with a Partial Take			
Prior to ACAM2000 (Week 8)	38	122.0 (88.9, 167.4)	813.3 (596.4, 1109.0)
Post ACAM2000 (Week 12)	37	140.8 (105.6, 187.8)	742.0 (589.6, 933.9)
Subjects with any Take			
Prior to ACAM2000 (Week 8)	76	117.2 (95.5, 143.8)	663.8 (540.6, 815.1)
Post ACAM2000 (Week 12)	75	198.3 (156.2, 251.7)	904.4 (766.2, 1067.6)
Subjects without a Take			
Prior to ACAM2000 (Week 8)	89	122.9 (99.8, 151.4)	687.1 (587.0, 804.3)
Post ACAM2000 (Week 12)	87	55.3 (44.1, 69.4)	365.5 (306.8, 435.5)

Source: Data were calculated by CBER statistical reviewer based on the Analysis Dataset (ADFA2.XPT and ADEFF2) of POX-MVA-006, STN125678-0.
Note: Subjects were vaccinated with ACAM2000 at Week 8, and antibody titers at Week 8 were before ACAM2000 vaccination. Week 12 was Peak Titer Visit for ACAM2000.

Reviewer's comments: *The high rate of no-take reactions was an unexpected study outcome. The proportion of subjects with any take and the degree of take attenuation were not consistent with those reported in POX-MVA-002 (refer to the appendix of this review) as well as literature report[9]. POX-MVA-002 evaluated the effect of previous vaccination with two doses of MVA-BN 28 days apart on skin lesions as well as antibody responses following vaccination with Dryvax at 84 days after the last dose of MVA-BN.*

In POX-MVA-002, prior vaccination with MVA-BN resulted in attenuation in skin lesions caused by Dryvax. However, there was no difference in take-rate between subjects who were previously vaccinated with MVA-BN (take rate 91%)

and subjects who were vaccinia naïve (take rate 100%). Although the difference in take rate could be possibly attributed to the different vaccines used in inducing take (Dryvax in POX-MVA-002 vs. ACAM2000 in the current study) and the intervals of the last MVA vaccination and Dryvax or ACAM2000 vaccination (84 days in POX-MVA-002 vs. 28 days in the current study), other factors such as vaccinators' bias and vaccination failure could not be excluded. Specifically:

1) *Operation (i.e., ACAM20000 vaccination in Group 1) bias could not be excluded due to the open-label design.*
2) *The takes (full and partial takes) among Group 1 subjects as reported in Listing 16.2.2.1 appear to be distributed in clusters. Statistical analyses also indicated that the take distribution among Group 1 did not appear to be randomly distributed.*
3) *Subjects regardless of their take type showed similar vaccinia specific antibody titers prior to ACAM2000 vaccination. However, after ACAM2000 vaccination, subjects without a take showed statistically significantly lower antibody titers than their pre-ACAM2000 vaccination titers, while subjects with a full take showed significant increase in antibody titers following ACAM2000 vaccination (Table 12). Theoretically, subjects who were previously vaccinated with MVA-BN should have amnestic immune responses following ACAM2000 vaccination. However, it appears that among subjects without a take (53.7% subjects, 87 out of 162 subjects) the expected amnestic antibody responses did not occur. There is no satisfactory explanation why subjects who did not have a take following ACAM2000 vaccination even had decreased vaccinia-specific antibody titers compared with that prior to ACAM2000 vaccination.*

An IR (#13) was sent to the applicant on 12 February 2019 regarding the apparent clustered distribution of take and lack of antibody response after vaccination with ACAM2000 among subjects without a take in Group 1. The applicant submitted its responses to STN125678/0.21 on 26 February 2019. The applicant agreed that there seemed to be clusters in terms of the unique subject ID but argued that it could be a random effect. Although the applicant agreed that even inactivated ACAM2000 should boost immune response in MVA-BN primed subjects, it argued that it would only occur when ACAM2000 were given parentally (e.g., subcutaneously, intramuscularly) but not via scarification. However, there is no evidence to support this argument.

The applicant also stated that the take pattern was less clustered in terms of ACAM2000 administration dates. This reviewer's analyses showed that take

distribution was clustered in terms of ACAM2000 administration dates stratified by calendar month or half-year (Table 13).

Table 13: Take Distribution among Group 1 Subjects in Terms of the Time Frame of ACAM2000 Administration (POX-MVA-006)

Time Frame of ACAM2000 Administration	% Subjects with any take, n/N (%)
By Calendar Month	
May 2015	0/1 (0%)
June 2015	1/8 (12.5%)
July 2015	6/10 (60%)
August 2015	5/6 (83.3%)
September 2015	3/3 (100%)
October 2015	3/6 (50%)
November 2015	3/3 (100%)
December 2015	3/3 (100%)
January 2016	2/2 (100%)
February 2016	2/3 (66.7%)
March 2016	0/2 (0%)
April 2016	2/6 (33.3%)
May 2016	0/1 (0%)
June 2016	2/6 (33.3%)
July 2016	2/7 (28.6%)
August 2016	7/19 (36.8%)
September 2016	13/29 (44.8%)
October 2016	1/4 (25%)
November 2016	1/6 (16.7%)
December 2016	7/14 (50%)
January 2017	24/54 (44.4%)
February 2017	1/1 (100%)
By Calendar Half-Year	
First half year of 2015 (May to June)	1/9 (11.1%)
Second half year of 2015 (July to December)	23/31 (74.2%)
First half year of 2016 (January to June)	8/20 (40%)
Second half year of 2016 (July to December)	31/79 (39.2%)
First half year of 2017 (January to February)	25/55 (45.5%)

Note: n=number of subjects with visible take; N=total number of subjects vaccinated with ACAM2000.

In addition, this reviewer has identified imbalances in take rate between study site 1 and site 2 regardless of analysis populations (Table 14).

Table 14: Comparison of Take Rates among Group 1 Subjects between Study Sites by Analysis Populations (POX-MVA-006)

	Site 1	**Site 2**
PPS-Take	N=77	N=88
% Subjects with full take (95% CI)	24.7 (15.6, 35.8)	21.6 (13.5, 31.6)
% Subjects with partial take (95%	32.5 (22.2, 44.1)	14.8 (8.1, 23.9)
% Subjects with no take (95% CI)	42.9 (31.6, 54.6)	63.6 (52.7, 73.6)
FAS	N=100	N=120
% Subjects with full take (95% CI)	22.0 (14.3, 31.4)	18.3 (11.9, 26.4)
% Subjects with partial take (95%	27.0 (18.6, 36.8)	13.3 (7.8, 20.7)
% Subjects with no take (95% CI)	43.0 (33.1, 53.3)	54.2 (44.8, 63.3)
Modified FAS	N=92	N=103
% Subjects with full take (95% CI)	23.9 (15.6, 33.9)	21.4 (13.9, 30.5)
% Subjects with partial take (95%	29.3 (20.3, 39.8)	15.5 (9.1, 24.0)
% Subjects with no take (95% CI)	46.7 (36.3, 57.4)	63.1 (53.0, 72.4)

Note: Modified FAS is defined as subjects in FAS who had take assessment data. N=number of subjects in the specified population.

Reviewer's comment: *Six subjects ((b) (6)) in Group 2 (vaccinia naïve subjects) had no take and all of them had undetectable vaccinia specific neutralizing antibody determined by both ELISA and PRNT following ACAM2000 vaccination, indicating vaccination failure. The applicant argued that lack of immune response and vaccination take following ACAM2000 vaccination among Group 1 subjects who were primed with MVA-BN could be due to "sterilizing immunity" induced by MVA-BN. Considering the clustering take distribution in terms of both the unique subject ID as well as ACAM2000 vaccination dates, and the imbalanced take rates between study sites 1 and 2, it is difficult to determine that lack of immune response and vaccination take following ACAM2000 vaccination among MVA-BN primed subjects was due to "sterilizing immunity" but not due to vaccination failure.*

We requested that BIMO inspect drug accountability lot (ACAM2000 lot, ACAM2000 preparation and storage following reconstitution, administration, and staff training). However, the inspector failed to obtain the requested information. Part of the requested information the inspector did not ask, and the other part was not made available by the DoD.

Based on the reasons stated above, this reviewer has no confidence in the validity of the take attenuation data and recommends not including the data in the product labeling.

6.1.11.3 Subpopulation Analyses

Reviewer's comment: *Subgroup analyses were not included in the original submission. The request for subgroup analyses was sent to the applicant on*

February 12, 2019 and the applicant submitted the analyses on February 26, 2019 to STN125678/0.21.

Since there are several issues with the take data, subgroup analysis of take attenuation will not be documented in this review.

Subgroup Analyses of PRNT GMT at Peak Visit

Subgroup analyses of PRNT GMT measured at Peak Visit in the PPS for immunogenicity stratified by age, sex, race, and ethnicity are presented in Table 15.

In all subgroups but one (American Indian or Alaskan Native subgroup), vaccinia specific neutralizing antibody titers measured by PRNT GMTs at Peak Visits among Group 1 subjects were non-inferior compared with Group 2. The 95% CI LBs for the corresponding GMT ratios (Group 1/Group 2) at Peak Visit were >0.5, the pre-specified non-inferiority margin for the primary endpoint of immunogenicity.

For the subgroup of American Indian or Alaskan Native, the PRNT GMT at Peak Visit among MVA-BN vaccinated subjects was lower than that among ACAM2000 vaccinated subjects. The clinical significance is unknown due to the limited number of subjects in this subgroup.

Table 15: Subpopulation analyses of Vaccinia Specific Neutralization Antibody Geometric Mean Titers (GMT) Determined by Plaque Reduction Neutralization Test (PRNT) Stratified by Age, Sex, Race and Ethnicity-PPS-IMM Population

Subgroup	Group 1 (N=185) GMT (95% CI) [n]	Group 2 (N=186) GMT (95% CI) [n]	Group 1/Group 2 GMT Ratio (95% CI)
Age (Years)			
18-24	160.6 (137.6, 187.5) [128]	91.3 (76.6, 108.8) [128]	1.8 (1.4, 2.2)
25-34	128.3 (96.0, 171.5) [49}	66.9 (52.4, 85.6) [50]	1.9 (1.3, 2.8)
35-44	198.9 (67.9, 582.1) [8]	101.8 (61.0, 169.7) [8]	2.0 (0.7, 5.7)
Sex			
Female	200.1 (144.2, 277.8) [36]	87.4 (60.9, 125.3) [28]	2.3 (1.4, 3.7)
Male	143.1 (123.3, 166.1) [149]	83.9 (72.0, 97.7) [158]	1.7 (1.4, 2.1)
Race			
American Indian or Alaskan Native	49.7 (17.6, 140.3) [7]	70.3 (29.5, 167.3) [4]	0.7 (0.2, 2.8)
Asian	128.0 (60.8, 269.3) [11]	106.8 (64.1, 178.1) [10]	1.2 (0.5, 2.8)
Black	226.2 (170.8, 299.5) [40]	60.0 (24.1, 85.1) [34]	3.8 (2.4, 5.8)
Native Hawaiian or Pacific Islander	274.8 (80.6, 937.1) [3]	74.6 (24.1, 231.2) [3]	3.7 (1.3, 10.8)
White	143.6 (120.7, 170.7) [108]	89.8 (75.5, 107.0) [121]	1.6 (1.3, 2.0)
Other	144.0 (101.5, 204.2) [16]	102.6 (56.7, 185.5) [14]	1.4 (0.7, 2.7)
Ethnicity			
Hispanic or Latino	149.1 (106.4, 208.9) [44]	82.0 (59.2, 113.7) [34]	1.8 (1.1, 2.9)
Not Hispanic or Latino	153.9 (132.9, 178.3) [141]	84.9 (72.7, 99.2) [152]	1.8 (1.5, 2.2)

Source: Adapted from Tables 4 and 5 (page 7-8), Response to IR 32, Module 1.11.3, STN125678/0.50

Note: N=number of subjects in the specified group; n=number of subjects in the specified subgroup with data available. PRNT GMTs below LLOQ (=20) were imputed as 1/2 LLOQ

6.1.11.4 Dropouts and/or Discontinuations

The overall dropout rate for this study was around 10%, similar to other vaccine studies. The major reasons for discontinuations were due to inability to comply with scheduled study visits. In addition, the results of analyses of the co-primary endpoints using FAS population were similar to those using PPS population, indicating that dropouts had no impact on the study conclusions.

6.1.12 Safety Analyses

6.1.12.1 Methods

All enrolled subjects (N=433) who received at least one vaccination were included in the safety analysis (FAS). In Group 1, all 220 subjects received the first MVA-BN vaccination, 208 subjects (94.5%) returned their first memory aid and also received the second MVA-BN vaccination, 193 subjects (92.8%) subjects returned their second memory aid, and 196 subjects (89.1%) received the ACAM2000 vaccine. In Group 2, all 213 subjects received the ACAM2000 vaccination and 200 subjects returned their memory aid.

Adverse events were coded using MedDRA version 20.0. Please refer to Section 6.1.7 for safety (solicited, unsolicited AEs, SAEs and AESIs) assessment.

6.1.12.2 Overview of Adverse Events

Summary of Adverse Events

An overview of all AEs during the clinical trial in the FAS is presented in Table 16.

Table 16: Overview of Adverse Events Experienced by Any Subject During the Clinical Trial-FAS Population

Category	Group 1 (N=220) n (%)	Group 2 (N=213) n (%)
At least one AE	209 (95.0)	209 (98.1)
AE ≥grade 3	24 (10.9)	64 (30.0)
SAE	5 (2.3)	3 (1.4)
AESI	7 (3.2)	4 (1.9)
AE leading to withdrawal	2 (0.9)	0 (0.0)
Death	0 (0.0)	0 (0.0)

Source: Adapted from Table 22 (page 122) of POX-MVA-006 CSR, STN125678/0, Module 5.3.5.1.
Notes: SAE=serious adverse event; AESI=adverse event of special interest (i.e., Cardiac related event).

Most subjects had at least one AE during the clinical trial (95% subjects in Group 1, and 98.1% subjects in Group 2). More subjects in Group 2 experienced grade ≥3 AEs within 28 days after ACAM2000 vaccination than Group 1 after any MVA-BN vaccination [24 subjects (10.9%) in Group 1, and 64 subjects (30.0%) in Group 2]. The higher grade 3 AEs among Group 2 subjects were driven by severe solicited injection-site and systemic adverse reactions (Table 17).

During the clinical trial, 8 subjects experienced SAEs [5 subjects (2.3%) in Group 1, and 3 subjects (1.4%) in Group 2]. A total of 11 subjects experienced AESIs [7 subjects (3.2%) in Group 1, and 4 subjects (1.9%) in Group 2]. These SAEs and AESIs are reviewed in detail in Sections 6.1.2.4 and 6.1.12.5, respectively.

Two AEs, both in Group 1, led to withdrawal from the second dose of MVA-BN. These two AEs also led to withdrawal from the trial and are discussed further in Section 6.1.12.7.

Solicited Adverse Events

The solicited injection-site and systemic AEs by treatment group and vaccination period during the 15-day follow-up period after each vaccination are summarized in Table 17.

Reviewer's comment: *Some solicited injection-site reactions were collected by investigators as unsolicited injection-site adverse events because study subjects failed to record them in diary card. These mis-classified unsolicited injection-site adverse events are reported as solicited injection-site reactions in this review and*

Table 17 reflects corrected data. Please refer to the Reviewer's comment under Unsolicited Adverse Events for details.

Table 17: Solicited Adverse Events during the 15-Day Follow-up Period after Each Vaccination–FAS

Adverse Event	Group 1 Dose 1 MVA-BN (N=208) n (%)	Group 1 Dose 2 MVA-BN (N=193) n (%)	Group 1 Dose 3 ACAM2000 (N=187) n (%)	Group 2 Dose 1 ACAM2000 (N=200) n (%)
Injection-Site AEs				
Any Pain	97 (46.6)	71 (36.8)	28 (15.0)	135 (67.5)
Grade 3 Pain	4 (1.9)	0 (0.0)	1 (0.5)	33 (16.5)
Any Erythema	61 (29.3)	56 (29.0)	116 (62.0)	191 (95.5)
Grade 3 Erythema	0 (0.0)	0 (0.0)	0 (0.0)	5 (2.5)
Any Swelling	21 (10.1)	22 (11.4)	46 (24.6)	138 (69.0)
Grade 3 Swelling	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Any Induration	24 (11.6)	13 (6.7)	45 (24.0)	132 (66.0)
Any Pruritus	29 (12.9)	20 (10.3)	111 (59.4)	179 (89.5)
Grade 3 Pruritus	2 (1.0)	0 (0.0)	1 (0.5)	18 (9.0)
Systemic AEs				
Any Pyrexia	3 (1.5)	1 (0.5)	2 (1.1)	3 (1.5)
Grade 3 Pyrexia	1 (0.5)	1 (0.5)	1 (0.5)	1 (0.5)
Any Headache	40 (19.2)	24 (12.4)	29 (15.5)	77 (38.5)
Grade 3 Headache	1 (0.5)	3 (1.6)	2 (1.1)	10 (5.0)
Any Myalgia	39 (18.7)	22 (11.4)	19 (10.2)	76 (38.0)
Grade 3 Myalgia	0 (0.0)	1 (0.5)	0 (0.0)	8 (4.0)
Any Chills	6 (2.9)	3 (1.5)	12 (6.4)	34 (17.0)
Grade 3 Chills	1 (0.5)	0 (0.0)	0 (0.0)	3 (1.5)
Any Nausea	12 (5.8)	10 (5.2)	13 (7.0)	40 (20.0)
Grade 3 Nausea	1 (0.5)	1 (0.5)	1 (0.5)	7 (3.5)
Any Fatigue	44 (21.2)	22 (11.4)	34 (18.2)	88 (44.0)
Grade 3 Fatigue	1 (0.5)	1 (0.5)	2 (1.1)	9 (4.5)
Any Malaise	29 (13.9)	14 (7.3)	29 (15.5)	66 (34.0)
Grade 3 Malaise	2 (1.0)	2 (1.0)	3 (1.6)	11 (5.5)

Source: Adapted from Table 15.3.1.2.1, Appendix 4, Module 1.11.3_Responses to IR13, STN125678/0.21.

Notes: Grade 3 pain= Spontaneously painful; Grade 3 erythema, induration or swelling=maximal diameter ≥ 100 mm; all other Grade 3 AEs= preventing routine daily activities; N=number of subjects in the specific group; n=number of subjects with an indicated event.

In Group 1, the proportions of subjects with any solicited injection-site AEs after the first and the second dose were generally similar. The most frequent solicited injection-site AEs after MVA-BN vaccination were pain and erythema, and a few grade 3 AEs were reported after the first dose of MVA-BN, and none after the second dose (Table 17). The median duration of the AEs after the first and the second dose of MVA-BN ranged from 3-6 days and 2-3.5 days, respectively.

Each of the solicited injection-site AEs were reported more often and more severe in Group 2 than in Group 1 including the subjects in Group 1 following

ACAM2000 vaccination (Table 17). The median duration of the AEs after ACAM2000 ranged from 3-10 days for Group 1 and 7-21 days for Group 2.

Reviewer's comment: *Only 15% of subjects in Group 1 after ACAM2000 vaccination experienced injection-site pain, which was relatively low.*

The most common solicited systemic AEs were fatigue (21.2% following the first dose of MVA-BN in Group 1, and 44% following ACAM2000 in Group 2) and headache (19.2% following the first dose of MVA-BN in Group 1, and 38.5% following ACAM2000 in Group 2)). Subjects in Group 2 following vaccination with ACAM2000 experienced more frequent and severe solicited systemic reactions except for pyrexia than subjects in Group 1 following either dose of vaccination including ACAM2000 (Table 17).

Less than 2% of subjects reported a Grade 3 systemic AE following any vaccination in Group 1, and the Grade 3 AEs appeared to be distributed similarly across vaccination periods. In comparison, up to 5.5% subjects (ranging from 1.0% to 5.5% depending on a specific AE) experienced a Grade 3 systemic AE in Group 2 (Table 17).

Most of the solicited systemic AEs resolved within 5 days. Duration of solicited systemic AEs was similar overall between groups and across vaccination periods.

Unsolicited Adverse Events

A summary of unsolicited AEs at a rate > 1% during the 28 day period (with a window of 28-35 days) after any vaccination by system organ class (SOC) and preferred term (PT) stratified by treatment group and vaccination period is presented in Table 18.

Table 18: Incidence of Unsolicited Adverse Events (>1%) by System Organ Class and Preferred Term (PT) during the 28-Day after Each Vaccination–FAS

Adverse Event	Group 1 Dose 1 MVA-BN (N=220) n (%)	Group 1 Dose 2 MVA-BN (N=208) n (%)	Group 1 Dose 3 ACAM2000 (N=196) n (%)	Group 2 Dose 1 ACAM2000 (N=213) n (%)
At Least One AE	**125 (57.3)**	**86 (41.3)**	**110 (56.1)**	**119 (55.9)**
Blood and Lymphatic System Disorders	**10 (4.5)**	**6 (2.9)**	**4 (2.0)**	**23 (10.8)**
Lymphadenopathy	10 (4.5)	6 (2.9)	3 (1.5)	23 (10.8)
Gastrointestinal Disorders	**2 (0.9)**	**8 (3.8)**	**6 (3.1)**	**9 (4.2)**
Food poisoning	1 (0.5)	1 (0.5)	2 (1.0)	3 (1.4)
Nausea	0 (0.0)	3 (1.4)	1 (0.5)	1 (0.5)
General Disorders and Administration Site Conditions	**60 (27.3)**	**26 (12.5)**	**6 (3.1)**	**13 (6.1)**
Axillary pain	0 (0.0)	0 (0.0)	0 (0.0)	5 (2.3)
Injection site erythema	2 (0.9)	2 (1.0)	1 (0.5)	0 (0.0)
Injection site nodule	43 (19.5)	16 (7.7)	1 (0.5)	2 (0.9)
Injection site pruritus	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)
Injection site warmth	13 (5.9)	10 (4.8)	1 (0.5)	0 (0.0)
Non-cardiac pain	3 (1.4)	0 (0.0)	0 (0.0)	2 (0.9)
Infections and Infestations	**32 (14.5)**	**27 (13.0)**	**34 (17.3)**	**29 (13.6)**
Nasopharyngitis	7 (3.2)	4 (1.9)	6 (3.1)	5 (2.3)
Pharyngitis	4 (1.8)	3 (1.4)	2 (1.0)	3 (1.4)
Upper respiratory tract infection	14 (6.4)	14 (6.7)	14 (7.1)	14 (6.6)
Injury, Poisoning and Procedural complications	**24 (10.9)**	**14 (6.7)**	**17 (8.7)**	**17 (8.0)**
Contusion	3 (1.4)	1 (0.5)	2 (1.0)	2 (0.9)
Laceration	5 (2.3)	1 (0.5)	4 (2.0)	1 (0.5)
Ligament sprain	4 (1.8)	2 (1.0)	1 (0.5)	2 (0.9)
Muscle strain	3 (1.4)	3 (1.4)	1 (0.5)	4 (1.9)
Skin abrasion	4 (1.8)	3 (1.4)	2 (1.0)	1 (0.5)
Investigations	**3 (1.4)**	**4 (1.9)**	**7 (3.6)**	**5 (2.3)**
AST increase	0 (0.0)	3 (1.4)	2 (1.0)	3 (1.4)
Metabolism and Nutrition Disorders	**1 (0.5)**	**4 (1.9)**	**2 (1.0)**	**0 (0.0)**
Musculoskeletal and Connective Tissue Disorders	**20 (9.1)**	**10 (4.8)**	**13 (6.6)**	**14 (6.6)**
Arthralgia	4 (1.8)	4 (1.9)	2 (1.0)	6 (2.8)
Back pain	7 (3.2)	0 (0.0	4 (2.0)	1 (0.5)
Pain in extremity	3 (1.4)	1 (0.5)	0 (0.0)	3 (1.4)
Nervous System Disorders	**10 (4.5)**	**4 (1.9)**	**7 (3.6)**	**7 (3.3)**
Headache	4 (1.8)	2 (1.0)	6 (3.1)	5 (2.3)
Psychiatric Disorders	**1 (0.5)**	**1 (0.5)**	**4 (2.0)**	**5 (2.3)**
Anxiety	1 (0.5)	0 (0.0)	3 (1.5)	1 (0.5)
Insomnia	0 (0.0)	1 (0.5)	1 (0.5)	3 (1.4)
Respiratory, Thoracic and Mediastinal Disorders	**7 (3.2)**	**4 (1.9)**	**9 (4.6)**	**6 (2.8)**
Nasal congestion	3 (1.4)	0 (0.0)	0 (0.0)	1 (0.5)
Oropharyngeal pain	4 (1.8)	2 (1.0)	1 (0.5)	1 (0.5)
Skin and Subcutaneous Tissue Disorders	**11 (5.0)**	**6 (2.9)**	**44 (22.4)**	**51 (23.9)**
Dermatitis contact	0 (0.0)	0 (0.0)	44 (22.4)	49 (23.0)

Source: Adapted from Table 15.3.1.4.1 in Appendix 4, Module 1.11.3_Response to IR 13, STN125678/0.21.
Notes: N=number of subjects in the specific group; n=number of subjects with an indicated event.

The proportion of Group 1 subjects who reported at least one unsolicited AE during the 28 days after the first dose of MVA-BN was similar to that of Group 2 subjects following vaccination with ACAM2000 (57.3% vs. 55.8%). The

proportion of subjects who reported any unsolicited AEs after the second dose of MVA-BN was slightly lower (41.3%) compared with that following the first dose (57.3%).

Unsolicited AEs by PT were generally balanced between the groups and across vaccination periods except for lymphadenopathy, injection/vaccination site reactions and dermatitis contact. Lymphadenopathy and dermatitis were more frequently reported in Group 2 after vaccination with ACAM2000 (10.8% and 23%, respectively) compared to Group 1 (4.5% after the first dose MVA-BN and 2.9% after the second dose MVA-BN for lymphadenopathy and none for dermatitis contact after the MVA-BN vaccinations). The rate of dermatitis contact in Group 1 after the ACAM2000 vaccination (22.4%) was similar as that in Group 2 (23%).

Injection-site nodule and warmth were more frequently reported among subjects vaccinated with MVA-BN (injection-site nodule: 19.5% and 7.7% after the first and second dose respectively; and injection-site warmth: 5.9% and 4.8% after the first and second dose respectively) than among subjects following vaccination with ACAM2000 in both Groups 1 and 2 (<1% for both events).

Reviewer's comment: *In the submission of STN125678/0, higher frequencies of unsolicited injection/vaccination site reactions were reported in Group 1 following MVA-BN vaccination. The proportions of subjects with injection/vaccination site nodule, erythema and warmth were 19.5%, 18.1% and 5.9%, respectively, following the first dose of MVA-BN, and 7.7%, 14.5% and 4.8%, respectively following the second dose of MVA-BN, while only 0.9% subjects in Group 2 reported injection/vaccination site nodules following vaccination with ACAM2000.*

Analysis of the subset of subjects with any unsolicited adverse events in the ADAE dataset showed that only one (Subject (b) (6) *) and four (Subjects* (b) (6) *) subjects experienced injection-site erythema and vaccination-site erythema, respectively, that had an onset day and end day beyond the window of 15 days after each vaccination for solicited adverse event, and all other injection-/vaccination-site erythema in this subset had an onset and end days within the surveillance window for solicited location reactions. An IR was sent to the applicant on February 12, 2019 for explanation.*

The applicant submitted its responses to STN125678/0.21 on February 26, 2019. The applicant states that in trial POX-MVA-006, the subjects were asked to record injection-site and systemic reactions on a diary card and then return the card at the next scheduled visit. In several instances, the investigator at the trial site noticed injection site reactions during the next visit, but those were not recorded by the subject in the memory aid. If this was the case, the investigator recorded these symptoms as unsolicited adverse events, in contrast to the subject's own reported solicited events that were carried over into the solicited

adverse events section of the case report forms. In such a scenario, adverse events that were specifically asked for as solicited AEs could be reported into the unsolicited AE section of the case report forms. The applicant submitted the updated analyses to STN125678/0.21 (Module 1.11.3, Tables 15.3.1.2.1 and 15.3.1.4.1 in Appendix 4). In these updated tables, all injection-site and vaccination-site (solicited and unsolicited) terms were harmonized to injection-site terms only and all injection-site reactions with an onset and end day within 15 days after any vaccinations were included in the solicited AEs.

Reviewer's comment: *The updated analyses are reflected in Tables 17 and 18.*

A summary of Grade ≥3 unsolicited AEs in 28 days after each vaccination by PT is presented in Table 19. The Grade ≥3 unsolicited AEs were similarly distributed between Groups (1.6% after MVA-BN and 2.6% after ACAM2000 in Group 1, and 3.3% after ACAM2000 vaccination in Group 2). There were no more than 2 grade 3 AEs after any vaccination period for any PT.

Table 19: Summary of Incidence of Grade ≥ 3 Unsolicited Adverse Events in 28 Days after Each Vaccination by Preferred Term-FAS

Adverse Event	Group 1 Dose 1 MVA-BN (N=220) n (%)	Group 1 Dose 2 MVA-BN (N=208) n (%)	Group 1 Dose 3 ACAM2000 (N=196) n (%)	Group 2 Dose 1 ACAM2000 (N=213) n (%)
At Least One AEs	**6 (2.7)**	**1 (0.5)**	**5 (2.5)**	**7 (3.3)**
Preferred Term				
Appendicitis	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)
Arthralgia	1 (0.5)	1 (0.5)	0 (0.0)	0 (0.0)
Back pain	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Contusion	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Dermatitis contact	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)
Dyspnea	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)
Food Poisoning	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)
Gastroenteritis	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)
Heat exhaustion	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Insomnia	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Medial tibial stress syndrome	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)
Pain in extremity	2 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)
Panic attack	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Photosensitivity reaction	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Pneumonia	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)
Tibia fracture	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)
Wrist fraction	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)

Source: Adapted from Table 29 of POX-MVA-006 CSR (page 136), STN125678-0, Module 5.3.5.1.

Notes: N=number of subjects in the specific group; n=number of subjects with an indicated event.

Reviewer's comment: *Appendicitis and tibia fracture reported in Group 1 were also SAEs. Please refer to Section 6.1.12.4 for detail.*

Subject (b) (6) *in Group 1 experienced severe dyspnea 10 days after the ACAM2000 vaccination. The event resolved the same day and was considered an AESI and possibly related to ACAM2000. Please refer to Section 6.1.14.5 for details.*

6.1.12.3 Deaths

None.

6.1.12.4 Nonfatal Serious Adverse Events

All nonfatal SAEs during this clinical trial are summarized in Table 20. A total of 8 SAEs were reported during the clinical trial, 2 of which occurred within one of the 29-day follow-up periods (both in Group 1, both at 24 days after the first MVA-BN vaccination). The remaining 5 SAEs occurred outside 28 days after vaccination, 3 each in Group 1 and Group 2.

Table 20: Summary of Serious Adverse Events in Study POX-MVA-006 – FAS

Subject	Preferred Term	Onset Day	Relatedness	Outcome
Group 1				
(b) (6)	Appendicitis	24	Unrelated	Recovered
	Suicidal ideation	(b) (6)	Unlikely	Recovered
	Alcohol poisoning	102	Unrelated	Recovered
	Peritonsillar abscess	72	Unrelated	Recovered
	Tibia fracture	24	Unrelated	Recovered
Group 2				
(b) (6)	Traffic accident	206	Unrelated	Ongoing
	Rhabdomyolysis	38	Unlikely	Recovered
	Hemorrhoids thrombosed	90	Unrelated	Recovered

Source: Adapted from Table 30 of POX-MVA-006 CSR (page 139), STN125678/0, Module 5.3.5.1.

The two SAEs that were assessed unlikely related by the applicant are summarized below:

- Subject (b) (6), an 18 year old male, was hospitalized due to suicidal ideation at (b) (6) days after ACAM2000 vaccination. He received his first and second dose of MVA-BN on (b) (6), respectively; and ACAM2000 on (b) (6). On (b) (6), he was hospitalized after having severe suicidal ideations and was diagnosed with adjustment disorder with depression. He was prescribed fluoxetine for depression and was discharged on (b) (6). On 7 February 2017, the subject had not had any further episodes of depression, and the event was considered resolved. The investigator considered the SAE to be unlikely related to study treatment.

- Subject (b) (6), a 19 year old male, received his ACAM2000 on (b) (6), and was hospitalized on (b) (6) due to severe rhabdomyolysis. The subject presented to the ER with pre-syncopal

symptoms during a strenuous Army Physical Fitness Test. The subject was dehydrated and undernourished at the time of attempting to complete the fitness test. Lab work performed in the ER and during hospitalization revealed a peak creatine kinase (CK) of 4,700 U/L (reference range: 55–170), and creatine kinase-MB isoenzymes (CK-MB) of 44.4 U/L (reference range: <16), and his creatinine elevated to a level of 2.9 mg/dL (reference range: 0.66–1.25). He was treated with IV fluid hydration and was discharged on (b) (6). On his last clinic visit on (b) (6), the subject had no complaints and his physical examination was unremarkable. Laboratory results had improved with normal chemistry, urinalysis, CK-MB of 10.1 U/L, and a slightly elevated CK of 379 U/L. The event was considered resolved and unlikely to be related to the vaccination.

Reviewer's comment: *This reviewer has reviewed the narratives of the SAE reports and concurs with the applicant's causality assessments.*

6.1.12.5 Adverse Events of Special Interest (AESI)

Subjects with Cardiac Related Sign and Symptoms

AESIs that occurred during the clinical trial are presented in Table 21. A total of 11 subjects experienced signs or symptoms that are suggestive of cardiac toxicity during the trial (7 subjects in Group 1 and 4 subjects in Group 2). None of the AESIs were considered serious, and 1 AESI was Grade 3 (a dyspnea in Group 1 that occurred 10 days after the ACAM2000 vaccination, considered possibly related to the vaccine).

Table 21: Adverse Events of Special Interest Reported in POX-MVA-006

Subject ID	AESI	Onset Day	Relatedness	Outcome
Group 1				
(b) (6)	Dyspnea	125 days/3rd dose	Unrelated	Resolved
	Dyspnea exertional	1 day/3rd dose	Possibly	Resolved
	Dyspnea exertional	28 days/2nd dose	Unlikely	Resolved
	Dyspnea	8 days/2nd dose	Unlikely	Resolved
	Chest pain	14 days/1st dose	Unlikely	Resolved
	Dyspnea	10 days/3rd dose	Possibly	Resolved
	Non-cardiac chest pain	5 days/1st dose	Unrelated	Resolved
Group 2				
(b) (6)	Dyspnea exertional	6 days/1st dose	Possibly	Resolved
	Non-cardiac chest pain	8 days/1st dose	Possibly	Resolved
	Chest discomfort	4 days/1st dose	Unrelated	Resolved
	Anxiety	2 days/1st dose	Unlikely	Resolved

Source: Adapted from Table 31 of POX-MVA-006 CSR (page 141), STN125678/0, Module 5.3.5.1.

Note: AESI onset day is recorded as days after a corresponding vaccination. For example, 125 days/3rd dose represents 125 days after the third dose of vaccination. Subjects in Group 1 received three doses of vaccination, two doses of MVA-BN at Day 0 (1st dose) and 28 (2nd dose) and a dose of ACAM2000 at Day 42 (3rd dose). Subjects in Group 2 received a single dose of ACAM2000 at Day 0 (1st dose).

Four AESIs were considered possibly related to the vaccine (2 each in Group 1 and Group 2). The four AESIs are summarized below:

- Subject (b) (6) (Group 1), a 30-year-old Caucasian male, experienced a non-serious, moderate exertional dyspnea one day after the ACAM2000 vaccination. The subject received his two doses of MVA-BN and one dose of ACAM2000 on (b) (6), respectively. On his visit on (b) (6), he reported that he had felt short of breath each morning during physical training since 13 October 2016. He denied any episodes of chest pain or short of breath on the visit day. There was no significant finding on clinical evaluation, and ECG and troponin were normal on (b) (6). The physician noted that the subject took sildenafil on (b) (6), and sildenafil has a known side effect of exacerbation of dyspnea. Follow up visit on (b) (6) showed normal echocardiogram and troponin. The event was considered resolved and possibly related to ACAM2000 due to the temporal association.

- Subject (b) (6) (Group 1), a 21-year-old male, received his two doses of MVA-BN on (b) (6), respectively, and ACAM2000 on (b) (6). On 22 September 2016, he experienced a severe dyspnea near the end of the two-mile run during physical training. He reported that he had to stop for 5-10 seconds to catch his breath, but then resumed and finished his run without further symptoms. The subject denied chest pain, pressure, or discomfort of any type, as well as no diaphoresis, nausea, or vomiting. The subject had no cardiac history noted and his physical examination was normal. On this same day, the event was resolved. On (b) (6), his echocardiogram and troponin were normal. The subject denied additional episodes of dyspnea on exertion during the two subsequent visits on (b) (6). The event was considered resolved and possibly related to ACAM2000 due to its temporal association.

- Subject (b) (6) (Group 2), an 18-year-old female, experienced a mild exertional dyspnea at 6 days after ACAM2000 vaccination. She received her ACAM2000 on (b) (6). On (b) (6), the subject presented to the protocol scheduled visit (Visit 2) and stated that she had been fatigued and it had been hard to breathe when doing physical training over the past 3 days. The subject had not had any shortness of breath or fatigue symptoms at any other time of the day. An echocardiogram and her troponin test were normal. She had not presented exertional dyspnea during the subsequent visits. The event was considered resolved and possibly related to the study treatment.

- Subject (b) (6) (Group 2), a 20-year-old male, experienced a moderate non-cardiac chest pain at 8 days after ACAM2000 vaccination. He received his ACAM2000 on (b) (6) . On 28 Apr 2016, the subject experienced moderate sternal chest pain, light-headedness, and a headache. He stated that prior to the onset of the symptoms, he had participated in a physical training test during which he had been doing sit-ups, push-ups, and a 2-mile run which he completed in about 15 minutes. Over the course of the day, his chest discomfort worsened, and he felt short of breath, to the point that walking short distances made him feel winded. He stated that the symptoms were worse in the afternoon and that he developed an intermittent cough. No positional changes (sitting, leaning forward, lying or standing) relieved or worsened his symptoms. On (b) (6) , the subject presented to Visit 3 and denied any symptoms, reporting that he had participated in physical training that morning without difficulties including no chest pain, shortness of breath, or headache. His electrocardiogram showed no significant changes from Screening, and his troponin was normal. The event was considered resolved and possibly related to the vaccination.

Reviewer's comment: *This reviewer has reviewed the narratives of the above reported AESIs and their case report forms (CRFs) and concurs with the applicant's causality assessments except for Subject* (b) (6) *as described below.*

Subject (b) (6) , a 19-year-old male, reported a mild dyspnea at 8 days after having received his second dose of MVA-BN. He received his first and second dose of MVA-BN on (b) (6) , respectively. On (b) (6) , the subject was seen at the clinic for a skin rash. The subject denied any dyspnea at this appointment, had no cardiac symptoms, and his respiratory system was documented as normal. On (b) (6) , the subject presented for his Visit 6 appointment, at that time he reported an episode of shortness of breath on 4 August 2016. Physical examination on this day showed no signs of respiratory distress. CRF showed ECG was normal but "Troponin I is missing". The Investigator considered the mild dyspnea unlikely related to study treatment.

On (b) (6) , the subject refused his scheduled ACAM2000 vaccination and withdrew consent, effectively withdrawing from the study.

Reviewer's comment: *Since there was no alternative explanation for the dyspnea and the close association of the event with the MVA-BN vaccination, the causal relationship of the event with MVA-BN could not be excluded based on the available information.*

Subjects with Abnormal Troponins and Abnormal Electrocardiograms (ECG)

No subject in Group 1 had an abnormal troponin following any vaccination. One subject ((b) (6)) in Group 2 had an asymptomatic abnormal troponin (0.08 ng/ml, baseline <0.05 ng/mL) at 15 days after ACAM2000 vaccination.

According to the assessment of Central Laboratory, in Group 1, 6 of 220 subjects (2.7%) who had normal ECG at baseline shifted to abnormal at two weeks after the first dose of MVA-BN (Visit 3), and 8 of 220 subjects (3.6%) who had normal ECG at baseline shifted to abnormal at two weeks after the third dose of vaccination (i.e., ACAM2000, Visit 9). Among the subjects with abnormal ECG after vaccination, three subjects had abnormal ECG at both Visit 3 and 9. These abnormal ECGs included the first degree of AV block (2 events), incomplete right buddle branch block (1 event), early repolarization (5 events), and prolonged QTCF (3 events). None was considered clinically significant by the Central Laboratory.

In Group 2, 2 of 213 subjects (0.9%) who had normal ECG at baseline shifted to abnormal at two weeks after ACAM2000 vaccination (Visit 3). The abnormal ECGs were early repolarization and prolonged QTCF which were assessed as not clinically significant.

Reviewer's comment: *It appears that more subjects in Group 1 had abnormal ECG at two weeks after the first dose of MVA-BN (2.7%) compared with subjects in Group 2 (0.9%) two weeks after ACAM2000. Since all the abnormal ECGs were considered not clinically significant by the applicant and this reviewer, the ECG change is unlikely a safety concern.*

6.1.12.6 Clinical Test Results

Variations in clinical laboratory tests were observed during the study, but no significant difference was reported across vaccination periods or between groups.

6.1.12.7 Dropouts and/or Discontinuations

Two AEs reported by two subjects (Subjects (b) (6)), both in Group 1, led to withdrawal from the vaccination as well as withdrawal from the trial, neither were considered related to the vaccine. The two events are described in Section 6.1.12.4 (Subject (b) (6)) and Section 6.1.12.5 (Subject (b) (6)) in this review.

6.1.13 Study Summary and Conclusions

6.1.13.1 Summary of Immunogenicity and Take attenuation

The co-primary endpoints of this study were to demonstrate the efficacy of MVA-BN by assessing non-inferiority of MVA-BN compared to ACAM2000 in terms of vaccinia-specific PRNT antibody titer at the Peak Visits (Day 42 for Group 1 and

Day 28 for Group 2), and by showing that vaccination with MVA-BN prior to scarification with ACAM2000 resulted in an attenuation of the take.

PRNT GMTs at Peak Visits for Group 1 and Group 2 were 152.8 (95%CI: 133.3, 175.0) and 84.4 (95%CI: 73.4, 97.0), respectively. The PRNT GMT ratio of Group 1/Group 2 was 1.8 (95% CI: 1.5, 2.2), which met the protocol specified non-inferiority margin of LB of 1-sided 97.5% CI > 0.5. The vaccinia specific neutralizing antibody titer elicited by two doses of MVA-BN administered at 28 days apart was considered non-inferior to that elicited by the U.S. licensed smallpox vaccine, ACAM2000.

Reviewer's comment: *While neutralizing antibodies are considered to contribute to the protection conferred by smallpox vaccines, the antibody level or titer correlated with protection against Variola is not defined. Epidemiological studies performed during the eradication of smallpox showed that vaccinated subjects exposed to the virus did not contract the disease despite an absence of measurable neutralizing antibody titers[10, 11], suggesting that memory immune responses played a critical role in protection of smallpox. The applicant concluded that MVA-BN performed better than ACAM2000 based on PRNT GMTs at Peak Visits. However, this reviewer could not conclude that the evaluated regimen of MVA-BN was more effective than ACAM2000 as the applicant claimed, since it is unknown whether the higher peak GMT translates to greater effectiveness in the short term, and the immune response elicited by replicating smallpox vaccines persists much longer than MVA-BN as described in Section 6.2 (Studies POX-MVA-005/-023), and it is unknown whether vaccination with MVA-BN induces long-lasting memory immune response.*

The median MLA caused by ACAM2000 in MVA-BN vaccinated subjects was 0.0 mm^2 (95% CI: 0.0, 2.0), and the median MLA caused by ACAM2000 in vaccinia naïve subjects was 76.0 mm^2 (95% CI: 70.0, 87.0). The lesion area reduction was 97.9% (95% CI: 96.6%, 98.3%), which met the protocol specified success criterion of 40% reduction.

Although the protocol specified success criterion regarding take attenuation was met, the issues with take attenuated described in Section 6.1.11.2 could not be explained by the applicant, and the take attenuation data were determined to be unreliable and not appropriate for inclusion in the product labeling.

The applicant's originally proposed indication did not include monkeypox. During the review of this submission, we received inquiries from external stakeholders in the US government regarding licensure of vaccines for prevention of monkeypox. Immune responses generated by vaccinia virus based vaccines have been proved to protect humans from smallpox. Since both monkeypox and variola viruses belong to the orthopoxvirus genus, and their structural proteins are over 96% identical[12], it is reasonable to believe that immune responses generated by vaccinia virus would prevent monkeypox. CBER determined that

immunogenicity data of MVA-BN obtained in humans together with the non-human primate data (monkeypox challenge studies) already submitted to BLA 125678/0 support the indication for prevention of monkeypox. Therefore, CBER recommended including the indication in the product labeling.

Reviewer's comment: *Please refer to the product reviewer's review regarding the protection of MVA-BN against monkeypox in non-human primate studies.*

6.1.13.2 Safety

Solicited Adverse Events

Subjects vaccinated with MVA-BN experienced fewer solicited injection-site reactions compared with subjects vaccinated with ACAM2000. The most common solicited injection-site reactions were injection-site pain and erythema. The percentages of MVA-BN recipients who experienced injection site pain and erythema were 46.6% and 29.3%, respectively, while percentages of ACAM2000 recipients who experienced injection-site pain and erythema were 67.5% and 95.5%, respectively.

The percentages of subjects who reported injection-site reactions following the first MVA-BN vaccination were slightly higher than the second dose of MVA-BN. After vaccination with ACAM2000, subjects previously vaccinated with two doses of MVA in Group 1 experienced less frequent injection-site reactions compared with vaccinia naïve subjects in Group 2.

There were few Grade 3 events reported among subjects who were vaccinated with MVA-BN, 4 subjects experienced Grade 3 injection-site pain (1.9%) and 2 subjects experienced Grade 3 injection-site pruritus (1.0%). In comparison, among ACAM2000 recipients in Group 2, 33 subjects (16.5%), 18 subjects (9.0%), 5 subjects (2.5%) and 1 subject (0.5%) reported Grade 3 injection-site pain, pruritus, erythema and swelling, respectively.

Within Group 1, median duration for most PTs was greater after ACAM2000 vaccination than it was after MVA-BN vaccination (median duration ranges: 3.0-6.0 days in period 1, 2.0-3.5 days in period 2, and 3.0-10.0 days in period 3). Median duration was longer for each PT for Group 2 compared to Group 1 after ACAM2000 vaccination (median duration range: 7.0-21.0 days in Group 2).

Similarly, subjects vaccinated with MVA-BN reported less often and less severe solicited systemic reactions compared with subjects vaccinated with ACAM2000. The most common solicited systemic reactions among MVA-BN recipients were fatigue (21.2%), headache (19.2%), myalgia (18.7%), malaise (13.9%) and nausea (5.8%). The most common solicited systemic reactions among ACAM2000 recipients in Group 2 were fatigue (44.0%), headache (38.5%), myalgia (38.0%), malaise (34.0%), nausea (20.0%) and chills 17.0%).

Percentages of subjects in Group 1 who reported any solicited systemic reactions following each MVA-BN vaccination and ACAM2000 vaccination were similar.

Fewer MVA-BN recipients reported Grade 3 solicited systemic reactions compared with ACAM2000 recipients. Among MVA-BN recipients, one subject (0.5%) each reported Grade 3 chills, fatigue, myalgia, nausea and pyrexia, 2 subject (1.0%) reported Grade 3 malaise and 3 subjects (1.6%) reported Grade 3 headache. In comparison, the Grade 3 solicited systemic reactions and the corresponding percentages reported by ACAM2000 recipients in Group 2 were: malaise (5.5%), headache (5.0%), fatigue (4.5%), myalgia (4.0%), nausea (3.5%), chills (1.5%) and pyrexia (0.5%).

Unsolicited Adverse Events

Approximately 50% subjects reported at least one unsolicited adverse event after each vaccination, which was similar between groups and across vaccination periods (Group 1: 57.3% and 41.3% after MVA-BN vaccination 1 and 2 respectively, and 56.1% after the ACAM2000 vaccination; Group 2: 55.9%).

The most common SOC in Group 1 was General Disorders and Administration Site Conditions (27.3% and 12.5% following the first and the second dose of MVA-BN respectively), Infections and Infestations (14.5% and 13.0% following the first and the second dose of MVA-BN respectively) and Injury, Poisoning and Procedural Complications (10.9% and 6.7% following the first and the second dose of MVA-B respectively). The most common SOC in Group 2 were Skin and subcutaneous Tissue Disorders (23.9%), Infections and Infestations (13.6%) and Blood and Lymphatic System Disorders (10.8%).

The majority of unsolicited AEs were Grade 1 (>82%). Proportions of intensity grades were similar between groups and across vaccination periods. Two Grade 3 unsolicited AEs were considered by the Investigator to be related to the vaccination. One subject in Group 1 experienced non-serious, severe dyspnea after the ACAM2000 vaccination, and the event resolved the same day. One subject in Group 2 experienced non-serious, severe photosensitivity reaction, and headache 9 days after the ACAM2000 vaccination, and the events resolved in 4 days.

Serious Adverse Events

A total of 8 SAEs were reported by 8 subjects during the clinical trial, 5 in Group 1 and 3 in Group 2. For the 5 SAEs in Group 1, 2 (appendicitis and tibia fracture) occurred at 24 days after the first MVA-BN vaccination and 3 (alcohol poisoning, peritonsillar abscess and suicidal ideation) occurred after ACAM2000 vaccination (at study days of 72 to 157), none of them were considered treatment related.

The 3 SAEs reported in Group 2 were hemorrhoids thrombosed, rhabdomyolysis, and traffic accident; none of them were considered treatment related.

Adverse Events of Special Interest

A total of 11 subjects experienced an AESI during this clinical trial, 4 of which were considered possibly related to the vaccine, 2 AESIs (dyspnea and exertional dyspnea) in Group 1, and 2 AESIs (exertional dyspnea and non-cardiac chest pain) in Group 2. None of these four subjects had abnormal ECG and troponin, and none were serious. No cases of suspected, probable, or confirmed myo/pericarditis were reported.

Deaths or Adverse Events that Led to Withdrawal

There were no deaths during this clinical trial. Two AEs (chest pain and tibia fracture) led to withdrawal from the vaccination as well withdrawal from the trial. Both AEs occurred in Group 1 and neither were considered related to the vaccine.

Clinical Laboratory Results

Shifts in hematology and biochemistry parameters were observed, however they were not clinically meaningful and they were similar across vaccination periods and between treatment groups. No abnormal Troponin I value was considered clinically significant.

6.1.13.3 Conclusions

Vaccinia specific neutralizing antibodies elicited by the evaluated regimen of MVA-BN were non-inferior compared to those elicited by the licensed replicating smallpox vaccine ACAM2000. Taken together with animal efficacy data, the clinical immunogenicity data provide reasonable basis to infer the effectiveness of MVA-BN for prevention of smallpox and monkeypox.

MVA-BN demonstrated an acceptable safety profile in vaccinia-naïve subjects.

6.2 Trial #2

POX-MVA-005: A partially randomized, partially double-blind, placebo-controlled Phase 2 non-inferiority study to evaluate immunogenicity and safety of one and two doses of MVA-BN smallpox vaccine in 18-55 year old healthy subjects

POX-MVA-023: An open-label Phase 2 study to evaluate immunogenicity and safety of a single MVA-BN booster vaccination two years after the last MVA-BN vaccination in former POX-MVA-005 vaccinees

Reviewer's comments: *Since POX-MVA-023 was the extension study of POX-MVA-005, these studies are reviewed and documented together.*

Please refer to Reviewer's comments under Section 2.5 regarding using these two studies to support licensure for a booster dose.

6.2.1 Objectives

6.2.1.1 POX-MVA-005

Primary Objectives

- To compare antibody response determined by ELISA between subjects with a history of smallpox vaccination who received one dose of MVA-BN (Group 4, smallpox vaccine experienced subjects) and subjects without a history of smallpox vaccination who received two doses of MVA-BN at 28 days apart (Group 1, smallpox vaccine naïve subjects)
- ECG changes and cardiac related events

Secondary Objects

- To compare the antibody response determined by ELISA among the four different vaccination groups
- To compare the neutralizing antibody response determined by PRNT among the four different vaccination groups
- To compare the four different vaccination groups with regard to safety and reactogenicity

6.2.1.2 POX-MVA-023

Primary Objectives

- To evaluate the immune response elicited by a booster vaccination with MVA-BN two years after the primary MVA-BN vaccination (one dose vs. two doses)

Secondary Objects

- To evaluate safety after a booster MVA-BN
- To evaluate persistence of anti-vaccinia antibody titers after two years in POX-MVA-005 study groups 1, 2 and 4 as well as following a booster dose

6.2.2 Design Overview

6.2.2.1 POX-MVA-005

POX-MVA-005 was a partially randomized, partially double-blind, placebo-controlled, phase 2 non-inferiority trial to evaluate immunogenicity and safety of one and two doses of MVA-BN in vaccinia virus naïve healthy subjects (Groups 1-3) and healthy subjects who were vaccinated with the first generation of smallpox vaccine (or vaccinia-experienced) (Group 4), 18-55 years of age. Subjects in Group 1-3 were randomly enrolled and treated under double-blind

conditions while subjects in Group 4 was not randomized and treatment was open-label.

- Group 1: Two doses of MVA-BN (1 x 10^8 TCID50/dose), SC on Day 0 and 28
- Group 2: One dose of MVA-BN (1 x 10^8 TCID50), SC on Day 0, and one dose of Placebo on Day 28
- Group 3: Two doses of Placebo, SC on Day 0 and 28
- Group 4: A single dose of MVA-BN (1 x 10^8 TCID50), SC on Day 0

Vaccinia-specific immune responses determined by ELISA and PRNT were evaluated 2 and 4 weeks after each vaccination and 6 months after the last vaccination.

Reviewer's comments: *It is unclear which smallpox vaccines were previously received by subjects in Group 4. However, based on the data provided in dataset SC.xpt it appears that the majority of subjects received their smallpox vaccination more than 25 years prior to study 005, and its likely that the vaccine was derived from the Lister strain of vaccinia as -005 was conducted in Germany.*

6.2.2.2 POX-MVA-023

POX-MVA-023 was an open-label extension study of POX-MVA-005 to evaluate the safety and immunogenicity of a single booster dose of MVA-BN in MVA-BN experienced subjects. The first 75 subjects each from Group 1 and Group 2 received a booster vaccination with MVA-BN approximately two-years (-2/+3 months) after their individual last MVA-BN vaccination. All boosted subjects were evaluated for booster effect of MVA-BN. All other subjects enrolled in Groups 1 and 2 after the required 75 subjects for each group were not boosted.

Additionally, POX-MVA-023 was also designed to provide 2-year follow-up immunogenicity data for subjects vaccinated with one or two doses of MVA-BN in POXMVA-005 as well as those subjects with a history of smallpox vaccination who received a single dose of MVA-BN in POX-MVA-005.

Vaccinia-specific immune responses determined by ELISA and PRNT were evaluated 1, 2, and 4 weeks, and 6 months after the single booster vaccination.

Reviewer's comments: *To distinguish the two populations vaccinated with different smallpox vaccines, in this review subjects who received the first generation of smallpox vaccines are referred to as vaccinia-experienced subjects, while subjects who received primary vaccination with MVA-BN are referred to as MVA-experienced subjects.*

6.2.3 Population

Main Eligibility Criteria for POX-MVA-005:

- Healthy male and female subjects between 18 and 55 years of age with no abnormal ECG findings and no active or history of cardiac disease
- Groups 1-3: Subjects with no history of known or suspected previous smallpox vaccination and no detectable vaccinia scar
- Group 4: Subjects with a history of previous smallpox vaccination (written documentation and/or typical vaccinia scar), and the most recent smallpox vaccination ≥5 years ago

Main Eligibility Criteria for POX-MVA-023:

- Subjects in Study POX-MVA-005 who received at least one dose of MVA-BN and completed the trial according to the protocol (Subjects in Group 3 were ineligible)

6.2.4 Study Treatments or Agents Mandated by the Protocol

6.2.4.1 Agent Mandated by the Protocols

- MVA-BN: It was provided in LF aliquots with a nominal virus titer of 1 x 10^8 $TCID_{50}$ /ml representing a titer range from 1 x 10^8 to 7.9 x 10^8 $TCID_{50}$/ml. The release titer was 2.5 x 10^8 to 7.9 x 10^8 $TCID_{50}$/ml. Therefore, a dose of 0.5 mL contained at least 1 x 10^8 $TCID_{50}$.

  POX-MVA-005: Lot #170505, and POX-MVA-023: Lot #0040707)

- Placebo: Tris buffer, 0.5 mL per dose (for POX-MVA-005 only, Batch #030505)

6.2.4.2 Dose and Administration

A dose of MVA-BN at 1 x 10^8 $TCID_{50}$ in 0.5 mL or a dose of placebo was administered SC in the non-dominant upper arm as indicated in Figure 2. Only a subset of subjects in Groups 1 and 2 of POX-MVA-005 was included and received a booster dose of MVA-BN in POX-MVA-023.

Fig 2: Treatment Schedules in POX-MVA-005 and POX-MVA-023

	POX-MVA-005 Phase		POX-MVA-023 Phase
Group	Vaccination (Week 0)	Vaccination (Week 4)	Vaccination (Year 2)
1	MVA	MVA	MVA
2	MVA	Placebo	MVA
3	Placebo	Placebo	Ineligible
4	MVA	None	None

6.2.5 Directions for Use

Please refer to Section 6.2.4 above.

6.2.6 Sites and Centers

Harrison Clinical Research GmbH, Albrechtstr. 43, 80636 Munich, Germany

6.2.7 Surveillance/Monitoring

For both POX-MVA-005 and POX-MVA-023, study subjects were screened, evaluated and monitored in a similar manner as described for Trial #1 (POX-MVA-006) under Section 6.1.7 except for that solicited AEs were collected for 7 days (instead of 15 days) after each vaccination. For POX-MVA-023, safety labs, urine analysis, troponin and ECG were performed only if clinically indicated during visits after vaccinations.

6.2.8 Endpoints and Criteria for Study Success

6.2.8.1 POX-MVA-005

Primary Endpoints:

- Vaccinia-specific seroconversion rate derived from the ELISA specific antibody titers two weeks after the last vaccination

Seroconversion based on ELISA titer was defined as an antibody titer of ≥50 in subjects who were seronegative (titer <50) at baseline, or a two-fold increase of the antibody titer for subjects with pre-existing antibody titer at baseline.

Reviewer's comment: *The applicant had no agreement with CBER prior to conducting this study that the primary endpoint based on ELISA seroconvervsion rates would support use of a single booster dose of MVA-BN in subjects who were previously vaccinated with smallpox vaccines. When we agreed to consider data from these studies in the BLA, we determined to consider analyses of PRNT GMTs primarily.*

- Cardiac related events and ECG change at any time during the study

Secondary Endpoints:

- Vaccinia-specific seroconversion rate derived from the ELISA specific antibody titers four weeks after the last vaccination
- Vaccinia-specific seroconversion rate derived from the PRNT specific antibody titers two and four weeks after the last vaccination
- SAEs possibly, probably or definitely related to the study vaccine at any time during the study
- Grade 3 or higher adverse reaction possibly, probably or definitely related to the study vaccine within 28 days after vaccination.
- Solicited AEs within 1 week (Days 0-7) after vaccination

Seroconversion based on PRNT titer was defined as an antibody titer of ≥6 in subjects who were seronegative (titer <6) at baseline, or a two-fold increase of the antibody titer for subjects with pre-existing titer baseline.

Reviewer's comments: *The cut-off for seropositivity derived from PRNT was defined as ≥20 in the clinical protocol (Section 16.1 Pages 16 of 66 and 48 of 66). In the study report, the cut-off was defined as ≥6. In section 11.4.2 (Statistical/Analytical Issues) of the study report, the applicant stated that "Seroconversion derived from PRNT specific antibody titers was defined as an antibody titer ≥6 for subjects seronegative at baseline and not as an antibody titer ≥20, as specified in the protocol." However, CBER assay reviewers cannot confirm the PRNT assay validation. Therefore, the data from this study could not be included in the product labeling.*

6.2.8.2 POX-MVA-023

Primary Endpoint:

- Peak booster rate: defined as percentage of subjects with either an antibody titer ≥ 50 in a vaccinia-specific ELISA for subjects with a titer <50 at baseline, or an increase of the antibody titer compared to the baseline titer for subjects with a pre-existing antibody titer, derived from the individual peak response at either one, two or four weeks after the booster vaccination

Reviewer's comment: *Similar to POX-MVA-005, the applicant had no agreement with CBER prior to conducting this study that the primary endpoint*

based on ELISA seroconvervsion rates would support use of a single booster dose of MVA-BN in subjects who were previously vaccinated with MVA-BN.

Secondary Endpoints:

- Percentage of subjects with either an antibody titer ≥ 6 in a vaccinia-specific PRNT for seronegative subjects at baseline, or an increase of the antibody titer compared to the baseline titer for subjects with a pre-existing antibody titer in the PRNT
- Kinetics and magnitude of the humoral immune response measured by ELISA and PRNT
- Any serious adverse events possibly, probably or definitely related to the study vaccine by the last study visit
- Unsolicited non-serious adverse events within 28 days after the booster vaccination
- Any Grade 3 or 4 AEs possibly, probably or definitely related to the study vaccine within 28 days after the booster vaccination
- Solicited adverse reactions within one week (Days 0-7) after the booster vaccination

Reviewer's comments: *Similar to POX-MVA-005, the cut-off for seropositivity derived from PRNT was ≥20 in clinical protocol, while the cut-off was ≥6 in the study report.*

6.2.9 Statistical Considerations & Statistical Analysis Plan

6.2.9.1 POX-MVA-005

Study Hypothesis and Sample Size Estimation

The sample size calculation was based on the primary endpoint vaccinia specific seroconversion rate derived from the ELISA specific antibody titers.

The null hypothesis was seroconversion rate among subjects with a history of smallpox vaccination (Group 4) was not statistically inferior to that among subjects without a history of smallpox vaccination (Group 1).

Suppose p1 were the seroconversion rate in Group 1 and p4 were the seroconversion rate in Group 4. The test on non-inferiority was applied for the following hypothesis:

H0: $p4 - p1 \leq \Delta$ versus H1: $p4 - p1 > \Delta$

where Δ was the non-inferiority margin and was chosen in this trial as 5%.

Based on the experience with MVA-BN in healthy subjects, it was anticipated that the seroconversion rate in healthy vaccinia-naive subjects would reach 98-100%.

Assuming a significance level of 5%, a power of 80% and expected seroconversion rates of 98% in both groups, this yielded a sample size of 175 subjects per group (700 subjects in total). In order to account for drop outs, at least 180 subjects per group were treated.

Statistical Methods and Multiplicity Adjustment

The hypothesis was tested based on an exact, unconditional test for binomial differences. In addition, an exact one-sided 97.5% unconditional confidence interval for the difference of proportions was calculated. If the lower limit of this confidence interval was greater than 5% (or equivalent the p-value of the non-inferiority test was less than 5%), then the null hypothesis was rejected (StatXact).

In order to limit the overall type 1 error to a nominal level of 5%, a hierarchical test procedure was chosen: The null hypothesis was tested on the measurements of samples collected two weeks after the last vaccination. Only if this comparison showed a significant result, the comparison of data of samples collected four weeks after vaccination was performed.

A secondary analysis with the neutralization assay specific seroconversion rates (from PRNT) was performed similar to the method described above.

In addition to the main comparison of Group 4 versus Group 1, secondary comparisons were also made among all other groups.

All statistical tests for secondary time points and comparisons were considered descriptive. Therefore, no adjustment for multiple testing was done.

6.2.9.2 POX-MVA-023

Statistical Considerations

The sample size available in this booster study was limited by the available sample size from POX-MVA-005. In POX-MVA-005 there were 168 and 170 subjects in the PPS in Groups 1 and 2, respectively. Based on the observation that about 78% subjects returned at the two year follow-up visit in earlier study POX-MVA-004, it was expected that approximately 130 subjects in both Group 1 and Group 2 would be available for screening.

POX-MVA-005 showed that peak seroconversion rates were 99.4% and 97.6% after the last MVA-BN vaccination in Group 1 and Group 4, respectively.

The primary endpoint was the peak booster rate (measured at either Week 1, 2 or 4). Assuming 97.6% of subjects in Group 1 showed either an increase in ELISA antibody titer (if a baseline titer was ≥50) or a titer ≥50 following the

booster vaccination, then the required sample size to maintain 80% power to observe a rate of at least 95% subjects with an increase in titer or a titer of ≥1:50 would be 58. Therefore, 75 subjects per group recruited would be sufficient to provide 60 evaluable subjects per group for the immunogenicity analysis.

Reviewer's comments: *For both POX-MVA-005 and POX-MVA-023, an antibody titer below the cut-off value (ELISA: 50, and PRNT 6) was imputed to 1.*

6.2.10 Study Population and Disposition

6.2.10.1 Populations Enrolled/Analyzed

POX-MVA005

- FAS Population: Subjects who received at least one vaccination (either MVA-BN or placebo)
- PPS Population: Subjects who received two injections (Groups 1–3) or one injection (Group 4), completed all protocol specified visits for immune response assays and adhered to all protocol conditions (without major protocol violations)

Reviewer's comments*: The primary endpoint analysis was on FAS population.*

POX-MVA-023

- Safety Population: Includes subjects who had any safety data
- FAS: Includes subjects who had baseline and any post vaccination immunogenicity data
- PPS: Includes subjects who completed all study visits according to the protocol without major violation

Reviewer's comments*: The primary endpoint analysis was on the FAS population. In this trial, the safety population and FAS contained the same subjects. So, safety analyses were performed on the FAS.*

6.2.10.1.1 Demographics

POX-MVA-005

Baseline characteristics of study subjects in POX-MVA-005 are presented in Table 22. In each group, there were more female than male subjects. As expected, smallpox vaccine naïve populations (Groups 1-3) were younger than smallpox experienced subjects (Group 4). There were no apparent differences among the four groups regarding gender and race.

Table 22: Demographic and Baseline Characteristics (Study POX-MVA-005)

	Group 1 (N=183)	Group 2 (N=181)	Group 3 (N=181)	Group 4 (N=200)	Total (N=745)
Age (years) Mean ± SD	 25.3 ± 5.0	 25.4 ± 4.4	 26.0 ± 5.1	 41.5 ± 7.6	 29.8 ± 9.1
Sex Male n (%) Female n (%)	 86 (47.0) 97 (53.0)	 69 (38.1) 112 (61.9)	 74 (40.9) 107 (59.1)	 85 (42.5) 115 (57.5)	 314 (42.1) 431 (57.9)
Race White n (%) Asian n (%) Black n (%) Others n (%)	 178 (97.3) 1 (0.5) 0 4 (2.2)	 176 (97.2) 2 (1.1) 1 (0.6) 2 (1.1)	 177 (97.8) 1 (0.6) 0 3 (1.6)	 198 (99.0) 0 1 (0.5) 1 (0.5)	 729 (97.9) 4 (0.5) 2 (0.3) 10 (1.3)

Source: Adapted from Table 14.1.3 of POX-MVA-005 CSR Section 14 (page 7-81) under Module 5.3.5.1.
N=number of subjects in the specified group; n=number of subjects in the specified subgroup.

All subjects in Group 4 had a confirmed history of smallpox vaccination with written documentation and/or typical vaccinia scar, except for five subjects who erroneously allocated into Group 4.

These five subjects had been allocated into Group 4 at screening, because of the existence of a vaccination scar. It was detected later that the scar in these subjects (numbers (b) (6)) was the result of a documented BCG vaccination.

Subject (b) (6) (26 years old) was erroneously allocated into Group 3, because of the subject's statement that she had not had a smallpox vaccination. Later the subject provided a document proving that she had been vaccinated against smallpox.

All six subjects were excluded from the PP (per protocol) population.

POX-MVA-023

Baseline characteristics of study subjects in POX-MVA-023 are presented in Table 23. The baseline demographics were similar across the groups except for age in Group 4 subjects who were expected older than subjects in Groups 1 and 2.

Table 23: Demographic and Baseline Characteristics (Study POX-MVA-023)

	Group 1 (N=92)	Group 2 (N=91)	Group 4 (N=121)	Total (N=304)
Age (years) Mean ± SD	 27.7 ± 5.8	 27.7 ± 4.5	 44.9 ± 6.6	 34.6 ± 10.2
Sex Male n (%) Female n (%)	 42 (45.7) 50 (54.3)	 36 (39.6) 55 (60.4)	 54 (44.6) 67 (55.4)	 132 (43.4) 172 (56.6)
Race White n (%) Asian n (%) Black n (%) Others n (%)	 91 (98.9) 1 (1.1) 0 0	 88 (96.7) 1 (1.1) 0 2 (2.2)	 120 (99.2) 0 1 (0.8) 0	 299 (98.4) 2 (0.7) 1 (0.3) 2 (0.6)

Source: Adapted from Table 15.1.2.2 of POX-MVA-023 CSR Section 15 (page 10-11) under Module 5.3.5.2.
N=number of subjects in the specified group; n=number of subjects in the specified subgroup.

6.2.10.1.2 Medical/Behavioral Characterization of the Enrolled Population

The targeted population was healthy subjects with no clinically significant findings in the
medical histories. All subjects complied with the inclusion/exclusion criteria. No clinically significant difference in medical/behavioral characterization between groups except for history of smallpox vaccination (All subjects in Group 4 had a history of smallpox vaccination).

As can be expected due to the discrimination concerning history of smallpox vaccination, the vaccinia-naïve population (Groups 1–3) in this study was younger (mean age 25.3, 25.4 and 26.0 years, respectively) than the vaccinia-experienced population (mean age 41.5 years in Group 4).

6.2.10.1.3 Subject Disposition

POX-MVA-005

Disposition of study subjects is presented in Table 24. The study screened 1322 subjects, and 753 subjects were eligible. Of the 753 subjects, 549 subjects were smallpox vaccine naïve and were randomized at 1:1:1 to Groups 1-3, and 204 subjects were smallpox vaccine experienced subjects and were allocated to Group 4. Eight subjects (3, 1, and 4 in Groups 2, 3, and 4, respectively) were withdrawn prior to vaccination because they were no long eligible after re-check of the eligibility criteria.

For the 545 subjects in Groups 1-3, all subjects received at least one injection and 529 subjects received two injections. In Group 4, all 200 subjects received the planned single dose MVA-BN. In total, 22 subjects (3.0%) discontinued the study prematurely. Of the 22 subjects, two (0.3%) discontinued the study due to an AE.

Table 24: Study Subject Disposition-Study POX-MVA-005

Disposition	Group 1 N (%)	Group 2 N (%)	Group 3 N (%)	Group 4 N (%)	Total N (%)
Subjects screened					1322
Subjects randomized	183	184	182	204	753[a]
Subjects received at least one injection	183 (100)	181 (100)	181 (100)	200 (100)	745 (100)
Subjects received two injections	180 (98.4)	174 (96.1)	175 (96.7)	NA	529 (97.1)
Discontinued from study	8 (4.4)	8 (4.4)	6 (3.3)	0	22 (3.0)
Adverse event	0	1 (0.6)	1 (0.6)	0	2 (0.3)
Subject's request	3 (1.6)	6 (3.3)	2 (1.1)	0	11 (1.5)
Subject incompliance	5 (2.7)	1 (0.6)	2 (1.1)	0	8 (1.1)
Lost to follow up	0	0	1 (0.6)	0	1 (0.1)

Source: Adapted from Table 14.1.2 of POX-MVA-005 CSR Section 14 (page 6) under Module 5.3.5.1.
[a] Eight subjects (3, 1 and 4 subjects in Groups 2, 3 and 4, respectively) failed eligibility criteria at re-check.
NA: Not applicable.
N: number of subjects.

POX-MVA-023

In total, 306 subjects were recruited into this study. Two subjects (0.7%) withdrew consent at screening and were not included in the study population. Of the 304 subjects enrolled in the study, 75 subjects (24.7%) in Group 1 and 77 subjects (25.3%) in Group 2 received one booster vaccination with MVA-BN, and 152 subjects (50.0%) (17 in Group 1, 14 in Group 2 and 121 in Group 4) received no vaccination and were included in the trial for evaluating antibody persistence.

Among the 152 subjects in Groups 1 and 2 who received a booster dose of MVA-BN, 148 subjects completed all follow up visits as planned. Four subjects (all in Group 1) prematurely terminated the study; one subject lost to follow-up and three subjects withdrew due to personal reasons. One subject each in Groups 1 and 2 had major protocol violations (both had urine protein >30 mg/dL). As a result, 70 subjects in Group 1 and 76 subjects in Group 2 were included in the PPS.

6.2.11 Efficacy Analyses

6.2.11.1 Analyses of Primary Endpoint(s)

POX-MVA-005

The primary endpoint was to test whether the ELISA derived seroconversion rate among the subjects who had a history of smallpox vaccinations and received one dose of MVA-BN (Group 4) was non-inferior to that among the vaccinia-naïve subjects who received two doses of MVA-BN (Group 1), at two weeks after the last MVA-BN vaccination. Seroconversion was defined as the antibody titers >= 1:50 in a vaccinia specific IgG ELISA for initially seronegative subjects or two-fold increase of the antibody titer compared to the pre-existing baseline titer for

subjects with a pre-existing antibody titer in the ELISA. The non-inferiority margin was pre-specified in the protocol at (-5%; +∞).

As shown in Table 25, for both the FAS and PPS populations, the confidence intervals for the difference of seroconversion rates between Group 4 and Group 1 exceeded the pre-defined margin at two weeks (-7.36%; +∞, for FAS; and -6.27%; +∞, for PPS). Therefore, the pre-specified success criterion was not met. Statistically, it could not be concluded that the seroconversion rate in Group 4 after a single booster MVA-BN vaccination was non-inferior to the seroconversion rate in Group 1 after two doses of the primary vaccinations with MVA-BN.

Reviewer's comment: *As mentioned above, we did not agree with ELISA seroconversion rate analysis as an acceptable primary endpoint analysis to support an effectiveness of a single dose of MVA-BN in individuals who were previously vaccinated with smallpox vaccines.*

Table 25: Non-Inferiority Comparison of Seroconversion Rate Derived from ELISA Specific Antibody Titers Two Weeks After the Last MVA-BN Vaccination Between Group 4 and Group 1

Population	Seroconversion Rate Group 1 n/N (%)	Seroconversion Rate Group 4 n/N (%)	Group 4 – Group 1	97.5% CI
FAS	174/176 (98.9%)	191/200 (95.5%)	-3.4%	-7.36%; +∞
PPS	166/168 (98.8%)	186/193 (96.4)	-2.4%	-6.27%; +∞

Source: Adapted from Tables 11-1 of POX-MVA-005 CSR, Section 11 (page 47), Module 5.3.5.1.
Seroconversion was defined as the antibody titers >= 1:50 in a vaccinia specific IgG ELISA for initially seronegative subjects or twofold increase of the antibody titer compared to the pre-existing baseline titer for subjects with a pre-existing antibody titer in the ELISA.
N=number of subjects in FAS or PPS population; n=number of subjects with seroconversion in the corresponding population.

POX-MVA-023

The primary endpoint was peak "booster rate" which was defined as percentage of subjects at any post vaccination visit with either an appearance of antibody titers ≥ 50 in a vaccinia-specific ELISA (for initially seronegative subjects) or an increase of the antibody titer compared to the baseline titer (for subjects with a pre-existing antibodies). The primary immunogenicity dataset was the FAS. The primary hypothesis was that the peak antibody response could be reactivated in subjects within each group by a single booster dose of MVA-BN to an observed level of at least 95%.

Reviewer's comment: *We do not agree with the applicant's definition of booster rate or that the booster response as measured by ELISA is acceptable for support effectiveness of a booster dose. Although there is no official definition of a booster response, seroconversion is generally defined as an antibody titer greater than, or equal to, the assay LLOD for subjects who were seronegative prior to vaccination, or four-fold increase in antibody titer for subjects with pre-*

existing antibody prior to vaccination. The antibody titer for demonstrating a booster response should be at least the same as the level for defining seroconversion.

The "booster rates" for each group at one, two and four weeks after the booster dose are presented in Table 26. In both Group 1 and Group 2, 100% of subjects showed an ELISA titer ≥ 50 or increase in ELISA titer at Peak Visit (two weeks after the booster). Therefore, the applicant claimed that the study met its primary endpoint of > 95% of subjects achieving an increase in vaccinia specific IgG titer of at least 50 from baseline in both groups.

In addition, there was no statistically significant difference between Groups 1 and 2 in booster rates at all the time points measured in the study (Table 26).

Table 26: "Booster Rates" Measured by ELISA at One, Two and Four Weeks After a Booster Dose of MVA-BN (POX-MVA-023, FAS Population)

Time Point	"Booster Rate" Group 1* n/N (%)	"Booster Rate" Group 2* n/N (%)	Group 1 – Group 2 % (95% CI)
One Week after Booster	75/75 (100.0)	76/77 (98.7)	1.3 (-1.2, 3.8)
Two Weeks after Booster	75/75 (100.0)	77/77 (100.0)	NA
Four Weeks after Booster	74/74 (100.0)	77/77 (98.7)	NA

Source: Adapted from Tables 9 of POX-MVA-023 CSR, Section 11 (page 52), Module 5.3.5.2.
*"Booster rate" was defined as percentage of subjects with an antibody titer ≥ 1:50 in a vaccinia specific IgG ELISA for initially seronegative subjects or an increase of the antibody titer compared to the pre-existing baseline titer for subjects with a pre-existing antibody titer in the ELISA. Subjects in Group 1 received two primary doses of MVA-BN at 28 days apart, subjects received one primary dose of MVA-BN, and subjects in both groups received a single booster dose at two years after the primary vaccination.
N=number of subjects in the population; n=number of subjects with a booster effect in the population.
NA=not applicable

Individual peak GMT titers measured by ELISA in Group 1 and 2 were 1822 and 1724, respectively. No significant differences were seen in the GMTs at peak response between the two groups (p = 0.665).

6.2.11.2 Analyses of Secondary Endpoints

POX-MVA-005

Comparison of ELISA based seroconversion rates at four weeks after the last MVA-BN vaccination between Group 4 and Group 1 was one of the secondary endpoints. The non-inferiority margin was pre-specified in the protocol at (-5%; +∞). Non-inferiority of seroconversion rates at four weeks after the last MVA-BN vaccination between the two groups was not demonstrated (One-sided 97.5 CI were [-10.5%; +∞], and [10.8%, +∞] for FAS and PPS, respectively).

The other secondary endpoint was to assess whether seroconversion rate determined by PRNT among Group 4 subjects was non-inferior to that among Group 1 subjects, at two or four weeks after the last MVA-BN vaccination. The non-inferiority margin was pre-specified in the protocol at (-5%; +∞).

Seroconversion rates, as determined by PRNT, at two and four weeks after the last MVA-BN vaccination are presented in Table 27.

Table 27: Seroconversion Rates Derived from PRNT at Two and Four Weeks After the Last MVA-BN Vaccination (FAS Population)

Time Points	Group 1 n/N (%)	Group 2 n/N (%)	Group 3 n/N (%)	Group 4 n/N (%)
Two weeks after the last MVA-BN	157/176 (89.2)	98/174 (56.3)	0/175 (0)	157/200 (78.5)
Four weeks after the last MVA-BN	153/178 (86.0)	83/175 (47.4)	0/177 (0)	139/199 (69.8)

Source: Adapted from Tables 14.2.2 of POX-MVA-005 CSR, Section 14 (page 35), Module 5.3.5.1.
Seroconversion was defined as the antibody titers >= 6 in a vaccinia specific PRNT for initially seronegative subjects or twofold increase of the antibody titer compared to the pre-existing baseline titer for subjects with a pre-existing antibody titer in the PRNT.
N=number of subjects in FAS dataset; n=number of subjects with seroconversion.

Reviewer's comments: *Similar results were also obtained in PPS population.*

Comparison of the seroconversion rates as measured by PRNT at both two and four weeks after the last MVA-BN vaccination showed significant differences between Group 1 and Group 4 for both FAS and PPS populations (Table 28). The LBs of 97.5% CIs exceeded the pre-defined margin of -5%. Therefore, it could not be statisitcally shown that the PRNT based seroconversion rate in Group 4 was non-inferior to that in Group 1 in both FAS and PPS populations at two and four weeks after the last vaccination.

Table 28: Non-Inferiority Comparison of Seroconversion Rate Derived from PRNT Antibody Titers Two and Weeks After the Last MVA-BN Vaccination Between Group 4 and Group 1

Population	Seroconversion Rate Group 1 n/N (%)	Seroconversion Rate Group 4 n/N (%)	Group 4 – Group 1	97.5% CI
Two Weeks after the Last Vaccination				
FAS	157/176 (89.2)	157/200 (78.5)	-10.7%	-18.2, +∞
PPS	149/168 (88.7)	152/193 (78.8)	-9.9%	-17.6, +∞
Four Weeks after the Last Vaccination				
FAS	153/178 (86.0)	139/199 (69.8)	-16.2%	-24.4, +∞
PPS	144/168 (85.7)	132/192 (68.8)	-16.9%	-25.5, +∞

Source: Adapted from Tables 11-4 and 11-5 of POX-MVA-005 CSR, Section 11 (page 54), Module 5.3.5.1.
Seroconversion was defined as the antibody titers >= 1:6 in a vaccinia specific PRNT titer for initially seronegative subjects or twofold increase of the antibody titer compared to the pre-existing baseline titer for subjects with a pre-existing antibody titer in the PRNT.
N=number of subjects in FAS or PPS population; n=number of subjects with seroconversion in the corresponding population.

POX-MVA-023

The secondary endpoint was “booster rates” determined by PRNT. The “booster rates”, defined as percentage of subjects with a PRNT GMT ≥ 6 for subjects who were seronegative prior to the booster or an increase of the antibody titer for subjects who had a pre-existing PRNT GMT >6 at baseline, for each group at one, two and four weeks after the booster dose are presented in Table 29. There was no statistically significant difference between Groups 1 and 2 in “booster rates” at all the time points measured in the study (Table 29).

Table 29: “Booster Rates” Measured by PRNT at One, Two and Four Weeks After a Booster Dose of MVA-BN (POX-MVA-023, FAS Population)

Time Point	“Booster Rate” Group 1* n/N (%)	“Booster Rate” Group 2* n/N (%)	Group 1 – Group 2 % (95% CI)
One Week after Booster	69/75 (92.0)	66/77 (85.7)	6.3 (-3.7, 16.2)
Two Weeks after Booster	74/75 (98.7)	74/77 (96.1)	2.6 (-2.5, 7.6)
Four Weeks after Booster	70/74 (94.6)	70/77 (90.9)	3.7 (-4.5, 11.9)

Source: Adapted from Tables 12 of POX-MVA-023 CSR, Section 11 (page 55), Module 5.3.5.2.
*”Booster rate” was defined as percentage of subjects with an antibody titer ≥ 6 in a vaccinia specific PRNT for initially seronegative subjects or an increase of the antibody titer compared to the pre-existing baseline titer for subjects with a pre-existing antibody titer in the PRNT. Subjects in Group 1 received two primary doses of MVA-BN at 28 days apart, subjects received one primary dose of MVA-BN, and subjects in both groups received a single booster dose two years after the primary vaccination.
N=number of subjects in the population; n=number of subjects with a booster effect in the population.
NA=not applicable

The individual peak titers measured by PRNT in Group 1 and 2 were 166 and 117, respectively. No significant differences were seen in the GMTs at peak response between the two groups (p = 0.113).

The applicant also assessed kinetics of antibody responses following the two doses of MVA-BN primary vaccination as well as following a booster dose of MVA-BN vaccination.

Kinetics and magnitude of the humoral immune response measured by ELISA and PRNT after the primary two doses of MVA-BN vaccination at 28 days apart are presented in Table 30. As shown in Table 30, at 6 months after the last dose of MVA-BN vaccination, vaccinia specific antibody titers reduced to below the assay limit for ELISA or close to the assay limit for PRNT. At 24 months after the last dose of MVA-BN, the antibody titers were undetectable for both the assays.

Table 30: Persistence of Anti-Vaccinia Antibody Geometric Mean Titer (GMT) at All Sampling Points from the Last Dose of Primary MVA-BN Vaccination (Group 1, Study POX-MVA-005, and -023, FAS Population)

Time Point	**ELISA GMT (95% CI)**	**PRNT GMT (95% CI) [n]**
Baseline (Week 0) (N=183)	**1.4 (1.2, 1.7)**	**1.1 (1.0, 1.2) [183]**
Time after Last Vaccination		
2 Weeks (n=176)	496 (432, 569)	46 (35. 59)]
4 Weeks (n=178)	329 (288, 375)	34 (26, 44)
6 Months (n=178)	28 (21, 38)	7 (6, 9)
24 Months (n=92)	23 (15, 36)	1.2 (1.0, 1.5)

Source: Adapted from Tables 9.5 and 9.6. STN125678/0.9_Response to FDA IR 8, Module 1.11.3.
Notes: M=Months; N=number of subjects in the population; n=number of subjects with data available.

The kinetics of antibody response after a booster dose of MVA-BN was assessed in subjects who received the to-be-licensed regimen of MVA-BN primary vaccination two years prior and subjects who received the first generation of smallpox vaccines over 25 years prior.

As shown in Table 31, vaccinia specific neutralizing antibody titer determined by PRNT reached peak titer of 125 at 2 weeks after a booster dose of MVA-BN and reduced gradually to 64 at 4 weeks and to 49 at 6 months after the booster in MVA-BN primed subjects (Group 1). The kinetics of the antibody responses following a booster dose of MVA-BN among subjects who previously received replicating vaccinia virus based smallpox vaccines (Group 4) was similar to that among the MVA-BN primed subjects. However, the decline of vaccinia specific antibody titers among Group 4 subjects appeared to be slightly slower than among the MVA-BN primed subjects with the Group 4 subjects returning to titers near baseline by 2 years.

Table 31: Persistence of Anti-Vaccinia Antibody Geometric Mean Titer (GMT) as Measured by PRNT After a Booster Dose of MVA-BN Vaccination in Vaccinia-experienced Subjects (Study POX-MVA-005, and -023, FAS Population)

Time Point	Group 1 (N=75) GMT (95% CI) [n]	Group 4 (N=200) GMT (95% CI) [n]
Baseline (Week 0)	**1.2 (1.0, 1.5) [75]**	**21.6 (16.3, 28.5) [200]**
Time after Booster MVA-BN		
1 Week	54 (37, 76) [75]	No data
2 Weeks	125 (90, 175) [75]	175 (140, 219) [200]
4 Weeks	64 (43, 96) [74]	144 (118, 177) [199]
6 Months	49 (32, 75) [71]	106 (89, 127) [198]
24 Months	No data	10.3 (7.2, 14.6) [121]

Source: Adapted from Tables 10.5. STN125678/0.9_Response to FDA IR 8, Module 1.11.3.
Notes: M=Months; N=number of subjects in the population; n=number of subjects with data available.

Reviewer's comment: *The data presented in Table 31 were most pertinent to our consideration of effectiveness of a single booster dose among individuals who were previously vaccinated with smallpox vaccines, as we conveyed to the applicant during the pre-BLA meeting. However, the assay validation issues precluded accepting the data to support the single booster dose.*

The similar kinetics of antibody response as determined by ELISA following the booster dose of MVA-BN among both Group 1 and Group 4 subjects was observed (Table 32). However, the magnitudes of antibody responses determined by ELISA between Group 1 and Group 4 were different from those determined by PRNT, which was likely because the reporter virus used in ELISA was MVA-BN. The results measured by the ELISA would favor Group 1 subjects who received three doses of MVA-BN.

Table 32: Persistence of Anti-Vaccinia Antibody Geometric Mean Titer (GMT) as Measured by ELISA After a Booster Dose of MVA-BN Vaccination in Vaccinia-experienced Subjects (Study POX-MVA-005, and -023, FAS Population)

Time Point	Group 1 (N=75) GMT (95% CI) [n]	Group 4 (N=200) GMT (95% CI) [n]
Baseline (Week 0)	**24 (15, 39) [75]**	**39 (29, 51) [200]**
Time after Booster MVA-BN		
1 Week	738 (597, 912) [75]	No data
2 Weeks	1688 (1382, 2063) [75]	569 (473, 684) [200]
4 Weeks	1255 (1029, 1531) [74]	452 (389, 526) [199]
6 Months	462 (381, 559) [71]	180 (149, 217) [198]
24 Months	No data	135 (112, 162) [121]

Source: Adapted from Tables 10.6. STN125678/0.9_Response to FDA IR 8, Module 1.11.3.
Notes: M=Months; N=number of subjects in the population; n=number of subjects with data available.

6.2.11.3 Subpopulation Analyses

POX-MVA-005

It is not meaningful to conduct subgroup analyses since the study did not meet the protocol specified primary endpoint and the results would be difficult to interpret.

6.2.11.4 Dropouts and/or Discontinuations

In POX-MVA-005, in total 22 or 3% subjects dropped out the study. The low dropout rate would not significantly affect antibody analyses. No subjects in POX-MVA-023 dropped out from the primary endpoint analysis.

6.2.11.5 Exploratory and Post Hoc Analyses

To support the use of MVA-BN in smallpox vaccine experienced subjects, the applicant conducted a post hoc analysis to evaluate whether a single dose of MVA-BN would be sufficient to boost the immune response in a previously smallpox vaccine-experienced population to the levels similar to those induced in smallpox vaccine-naive subjects who received two doses of MVA-BN administered 4 weeks apart.

A post-hoc, non-inferiority analysis of GMTs measured by vaccinia specific PRNT was performed between Group 4 (smallpox vaccine experienced subjects) and Group 1 (smallpox vaccine naive subjects) of study POX-MVA-005. However, post-hoc analyses on data from a study that failed to meet its primary endpoint generally are not used to support a claim of effectiveness. In addition, the primary analysis was not acceptable from a regulatory standpoint for supporting effectiveness of a single booster dose. Furthermore, the PRNT validation issue precluded making meaningful interpretation of the PRNT GMT data. Therefore, the data are not presented in this review.

6.2.12 Safety Analyses

6.2.12.1 Methods

Please refer to Section 6.2.7 for an overview of the safety assessment. Safety data were analyzed on the FAS population.

6.2.12.2 Overview of Adverse Events

Solicited and unsolicited AEs are presented in this section, while SAEs (including deaths) and AESIs are described in Sections 6.2.12.3 to 5 below.

POX-MVA-005

Solicited adverse events

Solicited adverse events were collected via diary cards for seven days after each vaccination. Diaries monitoring solicited adverse reactions were available from 182 subjects in Group 1, 179 subjects each in Groups 2 and 3 and 200 subjects in Group 4.

Solicited injection-site adverse events stratified by treatment group and severity is presented in Table 33. Across all the treatment groups, most solicited injection-site adverse reactions were mild (grade 1) to moderate (grade 2), and 0.5% to 3.3% subjects experienced severe (grade 3) solicited injection-site adverse reactions (Table 33). No grade 4 solicited injection-site adverse reactions were reported.

The proportions of subjects with injection-site reactions in vaccine treatment groups (Groups 1, 2 and 4) were higher than that in the placebo group (Group 3). Subjects who received two doses of MVA-BN (Group 1) experienced slightly more injection-site adverse reactions compared with subjects who received one dose of MVA-BN (Group 2 and Group 4) (Table 33).

Table 33: Solicited Injection-Site Adverse Events in Seven Days after Any Vaccination (POX-MVA-005, FAS Population)

Adverse Event	Group 1 (N=182)	Group 2 (N=179)	Group 3 (N=179)	Group 4 (N=200)
Injection-Site Pain				
Any Grade n (%)	166 (91.2)	155 (86.6)	37 (20.7)	167 (83.5)
Grade 1 n (%)	117 (64.3)	114 (63.7)	25 (14.0)	123 (61.5)
Grade 2 n (%)	48 (26.4)	41 (22.9)	12 (6.7)	41 (20.5)
Grade 3 n (%)	1 (0.5)	0 (0.0)	0 (0.0)	3 (1.5)
Injection-Site Erythema				
Any Grade n (%)	166 (91.2)	146 (81.6)	39 (21.8)	169 (84.5)
Grade 1 n (%)	55 (30.2)	87 (48.6)	37 (20.7)	71 (35.5)
Grade 2 n (%)	105 (57.7)	59 (33.0)	2 (1.1)	93 (46.5)
Grade 3 n (%)	6 (3.3)	0 (0.0)	0 (0.0)	5 (2.5)
Injection-Site Swelling				
Any Grade n (%)	149 (81.9)	103 (57.5)	10 (5.6)	149 (74.5)
Grade 1 n (%)	58 (31.9)	71 (39.7)	9 (5.0)	91 (45.5)
Grade 2 n (%)	89 (48.9)	32 (17.9)	1 (0.6)	57 (28.5)
Grade 3 n (%)	2 (1.1)	0 (0.0)	0 (0.0)	1 (0.5)
Injection-Site Induration				
Any Grade n (%)	162 (89.0)	146 (81.6)	5 (2.8)	155 (77.5)
Grade 1 n (%)	86 (47.3)	111 (62.0)	4 (2.2)	97 (48.5)
Grade 2 n (%)	75 (41.2)	35 (19.6)	1 (0.6)	57 (28.5)
Grade 3 n (%)	1 (0.5)	0 (0.0)	0 (0.0)	1 (0.5)

Source: Table 12-3 (page 67-68), POX-MVA-MVA-005 CSR, Module 5.3.5.1.
N=Total number of subjects in the corresponding group with diary card collected; n=number of subjects with adverse events; %=n/N x 100

A tabulated overview of solicited systemic adverse reactions stratified by treatment group and severity is presented in Table 34. Across all the treatment groups, most of the solicited systemic adverse reactions were mild or moderate, less than 3% subjects experienced grade 3 systemic adverse reactions, and no subject experienced grade 4 systemic adverse reactions (Table 34).

Table 34: Solicited Systemic Adverse Reactions in Seven Days after Any Vaccination (POX-MVA-005, FAS Population)

Adverse Event	Group 1 (N=182)	Group 2 (N=179)	Group 3 (N=179)	Group 4 (N=200)
Body Temperature				
Any Grade n (%)	18 (9.8)	21 (11.7)	10 (5.6)	10 (5.0)
Grade 1 n (%)	13 (7.2)	17 (9.5)	8 (4.5)	10 (5.0)
Grade 2 n (%)	5 (2.7)	4 (2.2)	2 (1.1)	0 (0.0)
Headache				
Any Grade n (%)	60 (32.9)	84 (46.9)	49 (27.4)	53 (26.5)
Grade 1 n (%)	39 (21.4)	57 (31.8)	35 (19.6)	41 (20.5)
Grade 2 n (%)	19 (10.4)	21 (11.7)	12 (6.7)	10 (5.0)
Grade 3 n (%)	2 (1.1)	6 (3.4)	2 (1.1)	2 (1.0)
Myalgia				
Any Grade n (%)	29 (15.9)	22 (12.3)	20 (11.2)	42 (21.0)
Grade 1 n (%)	25 (13.7	14 (7.8)	14(7.8)	33 (16.5)
Grade 2 n (%)	4 (2.2)	8 (4.5)	6 (3.4)	9 (4.5)
Nausea				
Any Grade n (%)	17 (9.3)	22 (12.3)	13 (7.3)	18 (9.0)
Grade 1 n (%)	13 (7.1)	12 (6.7)	8 (4.5)	16 (8.0)
Grade 2 n (%)	4 (2.2)	5 (2.8)	4 (2.2)	2 (1.0)
Grade 3 n (%)	0 (0.0)	5 (2.8)	1 (0.6)	
Fatigue				
Any Grade n (%)	68 (37.4)	59 (33.0)	55 (31.7)	78 (39.0)
Grade 1 n (%)	51 (28.0)	41 (22.9)	38 (21.2)	52 (26.0)
Grade 2 n (%)	16 (8.8)	14 (7.8)	14 (7.8)	21 (10.5)
Grade 3 n (%)	1 (0.5)	4 (2.2)	3 (1.7)	5 (2.5)

Source: Table 12-2 (page 66), POX-MVA-MVA-005 CSR, Module 5.3.5.1.
N=Total number of subjects in the corresponding group with diary card collected; n=number of subjects with adverse events; %=n/N x 100

Unsolicited Adverse Events

Most unsolicited AEs were in the SOC of General Disorders and Administrative Site Conditions (239 AEs in 23.6% of all subjects) followed by Infections and Infestations (166 AEs in 18.8% of all subjects). The proportion of subjects in the placebo group (Group 3) who experienced at least one AE of General Disorders and Administrative Site Conditions was lower compared with treatment groups (8.3% in Group 3 compared with 30.1%, 27.1% and 28.5% in Groups 1, 2 and 4, respectively). Otherwise the rates of unsolicited AEs experienced by the treatment and placebo groups were similar.

On a PT level, the most common unsolicited AEs were injection-site pruritus (151 in 17.7% of all subjects), nasopharyngitis (97 AEs in 12.3% of all subjects), and headache (45 AEs in 5.8% of all subjects). Group 3 had fewer incidences of injection-site pruritus than the treatment groups (2.2% subjects in Group 3 versus 23.5%, 19.9% and 24.5% in Groups 1, 2 and 4, respectively), but there was no such pattern for nasopharyngitis and headache.

Reviewer's comments: *Injection-site pruritus was not a solicited AE in this study.*

POX-MVA-023

This trial included 3 groups, however, only Groups 1 and 2 received a booster dose of MVA-BN under this study. Therefore, solicited and unsolicited AEs are presented for Groups 1 and 2 only.

Solicited Adverse Reactions

Solicited injection-site AEs are presented in Table 35. The proportions of injection-site reactions were similar between the two group, and only a few severe reactions were reported in this study (Table 35). No grade 4 solicited injection-site adverse reactions were reported.

Table 35: Solicited Injection-Site Adverse Reactions in Seven Days after Any Vaccination (POX-MVA-023, FAS Population)

Adverse Event	Group 1 (N=75)	Group 2 (N=77)
Injection-Site Pain		
Any Grade n (%)	58 (77.3)	64 (83.1)
Grade 1 n (%)	41 (54.7)	40 (51.9)
Grade 2 n (%)	16 (21.3)	21 (27.3)
Grade 3 n (%)	1 (1.3)	3 (3.9)
Injection-Site Erythema		
Any Grade n (%)	60 (80.0)	65 (84.5)
Grade 1 n (%)	31 (41.3)	30 (39.0)
Grade 2 n (%)	29 (38.7)	33 (42.9)
Grade 3 n (%)	0 (0.0)	2 (2.6)
Injection-Site Swelling		
Any Grade n (%)	51 (68.0)	48 (62.3)
Grade 1 n (%)	28 (37.3)	28 (36.4)
Grade 2 n (%)	23 (30.7)	18 (23.4)
Grade 3 n (%)	0 (0.0)	2 (2.6)
Injection-Site Induration		
Any Grade n (%)	58 (77.3)	58 (75.3)
Grade 1 n (%)	40 (53.3)	35 (45.5)
Grade 2 n (%)	18 (24.0)	22 (28.6)
Grade 3 n (%)	0 (0.0)	1(1.3)

Source: Table 15.5.3 (page 168-169), POX-MVA-MVA-023 CSR, Section 15, Module 5.3.5.2.
N=Total number of subjects in the corresponding group with diary card collected; n=number of subjects with adverse events; %=n/N x 100

Solicited systemic adverse reactions stratified by treatment group and severity is presented in and Table 36. Most of the solicited systemic adverse reactions were mild or moderate, and a few subjects experienced grade 3 systemic adverse reaction, and no subject experienced grade 4 systemic adverse reaction (Table 36).

Table 36: Solicited Systemic Adverse Reactions in Seven Days after Any Vaccination (POX-MVA-023, FAS Population)

Adverse Event	Group 1 (N=75)	Group 2 (N=77)
Body Temperature		
Any Grade n (%)	3 (4.0)	4 (5.2)
Grade 1 n (%)	2 (2.7)	3 (3.9)
Grade 2 n (%)	1 (1.3)	1 (1.3)
Headache		
Any Grade n (%)	19 (25.4)	25 (32.5)
Grade 1 n (%)	14 (18.7)	19 (24.7)
Grade 2 n (%)	3 (4.0)	5 (6.5)
Grade 3 n (%)	2 (2.7)	1 (1.3)
Myalgia		
Any Grade n (%)	17 (22.6)	19 (24.7)
Grade 1 n (%)	15 (20.0)	13 (16.9)
Grade 2 n (%)	1 (1.3)	5 (6.5)
Grade 3 n (%)	1 (1.3)	1 (1.3)
Nausea		
Any Grade n (%)	6 (8.0)	12 (15.6)
Grade 1 n (%)	5 (6.7)	10 (13.0)
Grade 2 n (%)	1 (1.3)	2 (2.6)
Fatigue		
Any Grade n (%)	22 (29.4)	27 (34.7)
Grade 1 n (%)	15 (20.0)	19 (24.7)
Grade 2 n (%)	5 (6.7)	8 (10.4)
Grade 3 n (%)	2 (2.7)	0 (0.0)

Source: Table 15.5.2 (page 158-159), POX-MVA-MVA-023 CSR, Section 15, Module 5.3.5.2.
N=Total number of subjects in the corresponding group with diary card collected; n=number of subjects with adverse events; %=n/N x 100

Unsolicited Adverse Events

The most frequently reported AEs were nasopharyngitis (26.7% subjects in Group 1 vs. 11.7% subjects in Group 2), headache (6.7% subjects in Group 1 vs. 9.1% subjects in Group 2), and injection-site warmth (4.0% subjects in Group 1 vs. 3.9% subjects in Group 2). Except for nasopharyngitis, the incidences of unsolicited AEs between the two groups were similar.

6.2.12.3 Deaths

No deaths occurred in the two studies.

6.2.12.4 Nonfatal Serious Adverse Events

Nonfatal SAEs are summarized in Table 37. Thirteen cases of SAEs in 13 subjects were reported from these studies, 11 cases in POX-MVA-005 and 2 cases (Subjects (b) (6)) in POX-MVA-023. None of the SAEs except for sarcoidosis in Subject (b) (6) was assessed by the applicant as related to the study vaccine.

Table 37: Summary of SAEs Reported in POX-MVA-005 and POX-MVA-023

Subject ID†	Adverse Event	AE Onset	Relationship to Study Vaccine*	Outcome
Group 1				
(b) (6)	Nervous breakdown	5 days after the second dose of MVA-BN	Unlikely related	Recovered without sequelae
	Tonsillectomy	5 months after the second dose of MVA-BN	Unrelated	Recovered without sequelae
	Sarcoidosis	10 weeks after the second dose of MVA-BN	Possibly related	Ongoing
	Gastroenteritis	4 months after the booster	Unrelated	Recovered without sequelae
	Concussion	5 months after the booster	Unrelated	Recovered without sequelae
Group 2				
(b) (6)	transient motoric hemiparesis on the right side	6 days after the first dose of MVA-BN	Unlikely related	Recovered without sequelae
	Rupture of extension tendon of the left 4th finger	25 days after the second dose of MVA-BN	Unrelated	Ongoing
Group 3				
(b) (6)	Salmonella enteritis	6 months after the second dose of MVA-BN	Unrelated	Recovered without sequelae
	Thyroidectomy for a benign cyst	3 months after the second dose of MVA-BN	Unrelated	Recovered with sequelae
	fracture of the right tibia and fibula	3 months after the second dose of MVA-BN	Unrelated	recovered with sequelae
	Colon carcinoma	10 weeks after the second dose of MVA-BN	Unrelated	recovered with sequelae
	Depression	9 weeks after the second dose of MVA-BN	Unlikely	Recovered without sequelae
Group 4				
(b) (6)	Rupture of the left tendon of the peroneus	4 months after MVA-BN vaccination	Unrelated	Recovered

Source: Summarized based on the narratives of SAEs reported in POX-MVA-005 CSR (page 81-82), Module 5.3.5.1, and POX-MVA-023 CSR (page 72), Module 5.3.5.2.
†The last three digits in Subject ID were the same for the same subjects in POX-MVA-005 and POX-MVA-023.
*Applicant's assessment

Subject (b) (6) was a 30 year-old male who suffered from arthralgia 10 weeks after the second vaccination with MVA-BN and reported fever up to 38°C and night sweat. Based on bronchoscopy and biopsy, the subject was diagnosed with

sarcoidosis. As the cause of sarcoidosis was unknown, the investigator and the applicant classified the event as an important medical condition and as possibly related to the study. The subject was treated with ibuprofen for a month, and the event was ongoing.

Reviewer's comments: *While the mechanistic link between MVA-BN and sarcoidosis is unclear, it is not possible to definitively exclude a causal relationship.*

6.2.12.5 Adverse Events of Special Interest

AESI was defined as any cardiac symptom, clinically significant ECG changes or cardiac enzymes elevated above upper limited normal (ULN). No subject with abnormal troponin following vaccinations in these studies was reported. A summary of the AESIs reported in POX-MVA-005 and POX-MVA-023 is presented in Table 38. A total of 20 cases were reported in 17 subjects (13 subjects in the phase of POX-MVA-005 and 4 subjects in the phase of POX-MVA-023), and most of cases were palpitations, tachycardia and sinus tachycardia. Among the 20 cases, 5 events were considered by the applicant as possibly related to the study vaccine. Two of these possibly related AESIs occurred in two female subjects in Group 2 (palpitation in Subjects (b) (6) and tachycardia in Subject (b) (6)), and the other three events occurred in Group 4 in one male (Subject (b) (6), 2 reports of palpitations) and one female subject (Subject (b) (6), tachycardia). All the events were recovered without sequelae.

Table 38: Summary of Adverse Event of Special Interest (AESI) Reported in POX-MVA-005 and POX-MVA-023

Subject ID†	Adverse Event	AE Onset	Relationship to Study Vaccine	Outcome
Group 1				
(b) (6)	Sinus tachycardia	14 days after the second dose of MVA-BN	Unlikely related	Recovered
	tachycardia	31 days after the second dose of MVA-BN	Unlikely related	Recovered
	palpitation	2 days after the first dose of MVA-BN	Unlikely related	Recovered
	palpitation	35 days after the booster dose of MVA-BN	Unrelated	Recovered
	palpitation	18 days after the booster dose of MVA-BN	Unrelated	Recovered
Group 2				
(b) (6)	palpitation	15 hours after the first dose of MVA-BN	Possibly related	Recovered
	sinus tachycardia	13 days after the second dose of MVA-BN	Unrelated	Recovered
	tachycardia	28 days after the second dose of MVA-BN	Possibly related	Recovered
	palpitation	6 months after the second dose of MVA-BN	Unlikely related	Ongoing
	palpitation	13 days after the booster dose of MVA-BN	Unrelated	Recovered
	non-cardiac chest pain	45 days after the booster dose of MVA-BN	Unrelated	Ongoing
Group 3				
(b) (6)	palpitation	23 days after the first dose of MVA-BN	Unrelated	Ongoing
Group 4				
(b) (6)	palpitation	2 events of palpitation at 3 and 5 months after MVA-BN vaccination	Unrelated	Recovered
	palpitation	3 events of palpitation occurred at 3 hours, 3 days and 3 months, respectively, after MVA-BN vaccination	Possibly related	Recovered
	tachycardia	6 months after MVA-BN vaccination	Unlikely related	Recovered
	tachycardia	A few hours after MVA-BN vaccination	Possibly related	Recovered
	nocturnal palpitation and pericardial effusion	Palpitation at 34 hours and pericardial effusion at 6 months, after MVA-B vaccination	Unlikely related	Unknown

Source: Summarized based on the narratives of AESIs reported in POX-MVA-005 CSR (page 83-85), Module 5.3.5.1, and ISS Section for POX-MVA-023 CSR (page 267-270), Module 5.3.5.3.
†The last three digits in Subject ID were the same for the same subjects in POX-MVA-005 and -023.

Subject (b) (6) in Group 4 experienced a chest pain in the evening prior to the follow up visit at approximately six months after the MVA-BN vaccination. The subject was examined one day after the follow up visit by a cardiologist who diagnosed a mild
pericardial effusion by ultrasound (196 days after the vaccination). The effusion had not changed per the assessment of the cardiologist two weeks later. The cardiologist reported that the effusion was not clinically significant and that no treatment was warranted. Because of the extended period (approx. 6 months) between vaccination and the event, the applicant considered the event unlikely related to the vaccine. The outcome was not reported.

A mild pericardial effusion was incidentally found in Subject (b) (6) (Group 3, placebo) in the echocardiogram on (b) (6) . A follow up echocardiogram on (b) (6) showed no abnormal findings. The event was not classified as an AESI because following extensive work-up the cardiologist concluded that the event was not clinically significant.

Reviewer's comments: *This reviewer has reviewed all the narratives of the reported AESIs in these two studies and concurs with the applicant's causality assessments.*

6.2.12.6 Clinical Test Results

There were no individual clinically significant abnormalities reported in the two studies.

6.2.12.7 Dropouts and/or Discontinuations

The total dropout rate was less than 3% (22 out of 745 subjects in POX-MVA-005, and 4 out of 152 subjects in POX-MVA-023 discontinued the studies). This low dropout rate would not significantly affect analyses of safety and the study conclusion.

Two of the 26 subjects were withdrawn due to adverse events. Subject (b) (6) (Group 2 in POX-MVA-005) experienced an SAE, transient motoric hemiparesis, six days after the first dose of MVA-BN. The event was assessed by the investigator as being unlikely related to study vaccine and possibly related to the underling medical condition (migraine accompagnée). Subject (b) (6) (Group 3, placebo) was incidentally detected a mild pericardial effusion which was assessed as not clinically significant by the cardiologist. Please refer to Sections 6.2.12.4 (Nonfatal SAEs) and 6.2.12.5 (AESIs) for details.

Reviewer's comment: *The transient motoric hemiparesis reported by Subject (b) (6) was considered likely related to her underlying medical condition migraine accompagnée by the applicant. The subject had suffered from migraine accompagnée 1-2 times a year since 1992. This reviewer concurs with the assessment.*

6.2.13 Study Summary and Conclusions

6.2.13.1 Antibody Response

The following results were obtained using a PRNT assay that was not sufficiently validated to support regulatory decision-making and an ELISA assay that is not considered sufficiently clinically meaningful to support smallpox or monkeypox vaccine effectiveness:

- Two doses of MVA-BN administered at 28 days apart in vaccinia naïve subjects appeared to be immunogenic as determined by vaccinia specific PRNT and ELISA. The vaccinia specific antibody titers reached to the peak at two weeks after the last dose of MVA-BN, declined to the assay cut-off levels at six months after the last dose of MVA-BN and were not detectable at two years after the primary vaccination series.

- One dose of MVA-BN, administered at two years after the primary vaccination with MVA-BN, appeared to be able to boost vaccinia specific antibody responses in the MVA-BN experienced population. The antibody titers after the booster dose of MVA-BN in MVA-BN primed subjects were higher and lasted longer than those after the primary vaccination with MVA-BN. The antibody titers at six months after the booster dose of MVA-BN were still around 10-fold higher than the LLOD of the assay as determined by both ELISA and PRNT.

- One dose of MVA-BN, administered up to 25 years or more after vaccination with replicating vaccinia virus based smallpox vaccines, appeared to be able to boost vaccinia specific antibody responses in these vaccinia experienced subjects. However, the vaccinia specific neutralizing antibody titers declined rapidly back to or below baseline titers within 2 years [21.6 (95% CI: 16.3, 28.5) prior to the booster vs. 10.3 (95% CI: 7.2, 14.6) at two years after the booster].

The proposed primary endpoint, seroconversion rates determined by ELISA, was not intended, nor accepted by us, to support use of a single dose of MVA-BN in smallpox vaccine experienced individuals. In addition, PRNT immunogenicity data generated from these two studies cannot be accepted to support licensure because of the assay validation issues identified during review. Therefore, insufficient data are available to support effectiveness of a single booster dose in smallpox vaccine experienced individuals.

6.2.13.2 Safety

The safety profile of MVA-BN among the subjects assessed in these two trials is acceptable. The proportion of subjects with any AE was higher among subjects who received MVA-BN than that of subjects who received placebo. The most common solicited injection-site adverse reactions were pain, erythema, swelling and induration (each around 80%), and the most common solicited systemic

adverse reactions were fatigue and headache with incidence rates around 30%. Subjects who received a second dose of MVA-BN or a booster dose of MVA-BN tended to have fewer injection-site adverse reactions.

No deaths were reported in these studies. One case of nonfatal SAE (Sarcoidosis) was reported by one subject and was considered possibly treatment related. No clinically significant AESIs were reported in these studies.

6.3 Trial #3

POX-MVA-013: A Randomized, Double-Blind, Placebo-Controlled, Phase 3 Trial to Evaluate Immunogenicity and Safety of Three Consecutive Production Lots of MVA-BN Smallpox Vaccine in Healthy, Vaccinia-Naïve Adults

6.3.1 Objectives (Primary, Secondary)

The primary objective of this study was to assess the consistency of 3 consecutively produced MVA-BN lots. Secondary objectives included the assessment of cardiac AESIs and other uncommon adverse reactions compared to placebo and to collect vaccinia-specific humoral immune response data.

Reviewer's comment: *Reactogenicity was not listed as a study objective. However, reactogenicity data (solicited and unsolicited adverse events) were collected for 7 days after each vaccination and solicited and unsolicited adverse events were included in the secondary endpoints. Since this trial was the largest MVA-BN clinical trial with a placebo control, these data serve as the primary source of reactogenicity labeling information in smallpox vaccine naïve individuals.*

6.3.2 Design Overview

Study POX-MVA-013 was a randomized, double-blind, placebo-controlled Phase 3 lot consistency study in healthy, vaccinia naïve subjects which took place from 18 Mar 2013-23 May 2014. Four thousand subjects were randomized into four study groups (1:1:1:1 via block randomization) to receive two doses, at weeks 0 and 4, of MVA-BN from 1 of 3 consecutive lots or placebo (Trish buffered saline). Subjects were screened up to 28 days prior to enrollment (day -28 to -1) and first study vaccination. Subjects were seen for follow-up visits 2 weeks after each vaccination (week 2 and week 6), as well as at week 8 and 30 for additional adverse event assessment. Solicited AEs were collected via diary on days 0-7 after each vaccine dose and unsolicited AEs were collected for 28 days after each dose. Immunogenicity data (assessed by PRNT assay for primary analyses) was collected at baseline and 2 weeks after the second vaccine dose (week 6). Equivalence of PRNT titers from each lot was demonstrated if mean log10 titers fell within a pre-defined margin of equivalence and if GMT ratios between groups were similar. ECG and troponin I assays were collected at baseline and 2 weeks after the first immunization (week 2). Cardiac related signs

and symptoms were followed up from the date of the first vaccination throughout the study. The total study duration was 7 months.

Reviewer Comment*: In general, the design of this study for the purpose of assessing the consistency of immunogenicity across vaccine lots was appropriate. Minor issues with the strategy for imputation of PRNT values were identified and required re-calculation of study endpoints by the applicant (see section 6.3.8 for more details). .*

6.3.3 Population

Subjects in this study were healthy, 18-40 year old men and women with BMI between 18.5 and 35, and baseline safety laboratory values within normal limits (including WBC, ANC, hemoglobin, platelets, calculated creatinine clearance; bilirubin, AST, ALT and alkaline phosphatase ≤1.5 x ULN, troponin I ≤ 2 x ULN and ECG without clinically significant abnormalities, who agreed to use approved contraceptive methods until at least 28 days after last vaccination.

Subjects were excluded from enrollment if they met the following criteria:

- previously received a smallpox or other poxvirus vaccine
- in the military before 1991 or after 2003,
- pregnant or breastfeeding
- Active infection or history of any serious medical condition, including autoimmune, immunodeficiency, hematological, pulmonary, neurological, cardiovascular, gastrointestinal or uncontrolled mental health disorders
- At risk for ischemic heart disease (IHD) due to either immediate family member with IHD before the age of 50 or per the National Cholesterol Education Program's risk assessment tool (if score > 10%)
- History of allergy to vaccine components (tris buffer, egg, aminoglycosides) or anaphylaxis to any vaccine
- Received a vaccine within 14 (inactivated vaccines) or 30 (live attenuated vaccines) days,
- Received immunosuppressive therapy or blood product within 3 months of vaccine administration.

A total of 5357 subjects were screened and 4005 subjects were enrolled.

6.3.4 Study Treatments or Agents Mandated by the Protocol

Study subjects received the following products by subcutaneous injection as per randomized study group assignment:

- Group 1- two doses (day 0 and 28-35) of 0.5 mL LF MVA-BN vaccine (lot C00001)
- Group 2- two doses (day 0 and 28-35) of 0.5 mL LF MVA-BN vaccine (lot C00002)

- Group 3- two doses (day 0 and 28-35) of 0.5 mL LF MVA-BN vaccine (lot C00003)
- Group 4- two doses (day 0 and 28-35) of 0.5 mL Placebo vaccine (tris-buffered saline)

Each dose of liquid frozen MVA-BN vaccine contains a virus titer around 1 x 10^8 $TCID_{50}$.

Reviewer Comment: *Variation in virus titer occurred over the duration of the study within lots. Lot C00001 ranged from 1.8-3.6 x 10^8 $TCID_{50}$ (most variable), Lot C00002 ranged from 1.3-1.8 X10^8 $TCID_{50}$ and Lot C00003 ranged from 1.6-1.9 x 10^8 $TCID_{50}$.*

6.3.5 Directions for Use

Refer to section 6.3.4.

6.3.6 Sites and Centers

Thirty-four sites across the United States were used for this trial.

6.3.7 Surveillance/Monitoring

Subjects were screened, evaluated and followed as described in Table 39. After each vaccine dose, subjects were given a diary, ruler and thermometer and asked to record findings/symptoms (including daily temperature, local and systemic reactogenicity symptoms as well as any adverse events) and severity of those symptoms for 7 days from each vaccination and diaries were reviewed at each follow up visit by clinical trial site staff and input into the electronic CRF for each subject.

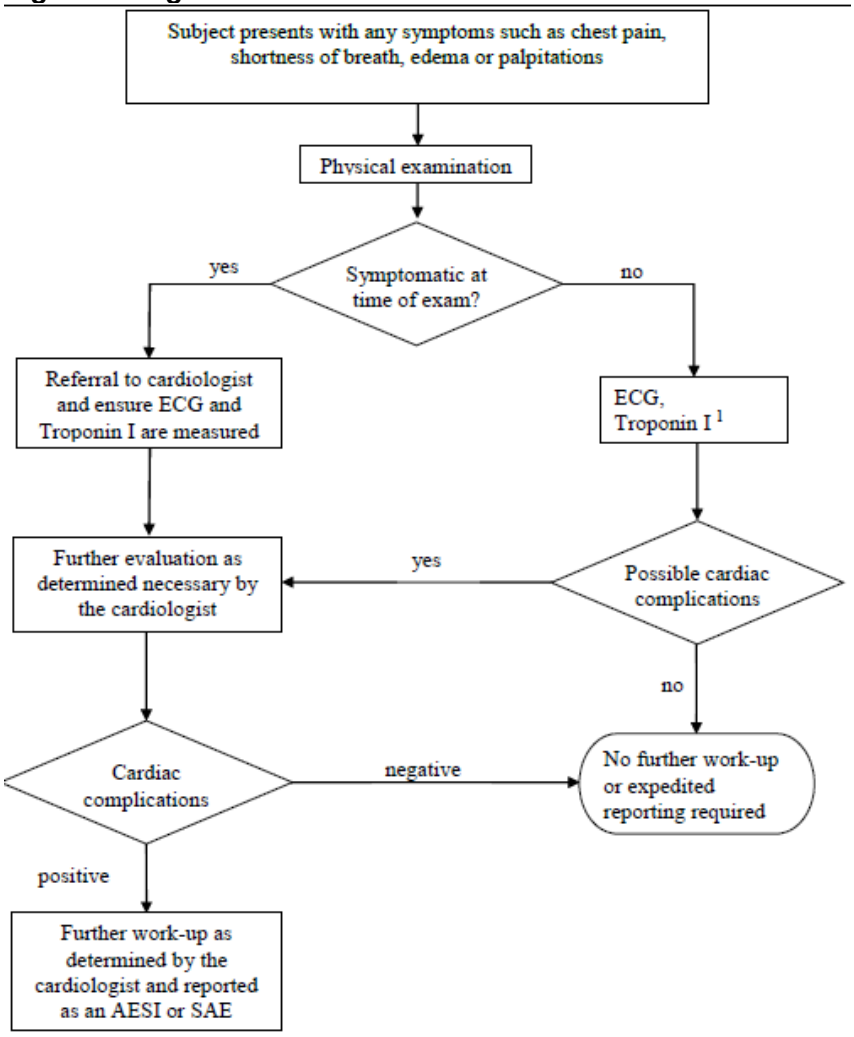
Table 39: Schedule of Study Procedures in POX-MVA-013

Visit (V)	Screen	V1	V2	V3	V4	V5	FU Phone
Day/ Visit + Day	-28- -1	0	V1 + 12-16	V1 + 28-35	V3 + 12-16	V3 + 28-35	V3 + 182-210
Target Week	-4	0	2	4	6	8	30
Informed Consent	X						
Check Incl/Excl Criteria	X	X					
Check Withdrawal Criteria				X			
Medical History	X						
Assessment for previous smallpox vaccination including check for a scar	X						
Complete Physical Exam	X						
Evaluation of Vital Signs	X	X	X	X	X	X	(X)
Calculate Individual Cardiac Risk Factors	X						
Evaluation of Family Cardiac Risk Factors	X						
Recording of Baseline Signs and Symptoms	X						
Target Physical Exam incl. auscultation of the heart and lung		X	X	X	X	X	(X)
ECG	X		X		(X)		
Recording of Prior and Concomitant Medication	X	X	X	X	X	X	
Counseling on Pregnancy Avoidance for Women of Child-bearing Potential (WOCBP)	X	X		X			
AE/SAE/AESI Recording		X	X	X	X	X	X
Pregnancy test for WOCBP	X	X		X		X	
Safety Labs	X		X		X		(X)
Total, HDL and LDL Cholesterol	X						
Troponin I	X		X		(X)		
Antibody analysis		X			X		
Vaccine Administration and Subject Observation (>30 min)		X		X			
Recording of Immediate AEs		X		X			
Handout of Memory Aid			X		X		
Examination of Injection Site			X		X		

(X)= will be performed only if clinically indicated
Source: Original BLA 125678/0; Adapted from POX-MVA-013, Clinical Study Report, p.32-33

Cardiac AESIs, which included myocarditis and pericarditis in this study, as well as AEs and SAEs, were recorded at every study visit. Myocarditis and pericarditis were defined per the CDC's 2003 MMWR report: Cardiac Related Events During the Civilian Smallpox Vaccination Program---United States, 2003. Subjects who developed any cardiac signs or symptoms concerning for possible myocarditis/pericarditis during the study received additional evaluation, including referral to a cardiologist, ECG and troponin I. Refer to the algorithm Fig 3.

Figure 3: Algorithm for Assessment of Cardiac Events in POX-MVA-013



Source: Original BLA 125678/0; POX-MVA=013 Appendix 16.1.1 Study Protocol and Amendments, p.49

Reviewer Comment: *While all subjects had routine collection of ECG and troponin I data after the first dose of vaccine, ECG and troponin I were only collected after the second dose of MVA-BN (week 6) if the subject demonstrated cardiac symptoms, such as chest pain or dyspnea. It was agreed (by CBER and the applicant) during the IND phase review of this study protocol that routine troponin and ECGs could be eliminated following the second dose of MVA-BN (as had been performed in several previous studies), as no clinical myopericarditis cases had been detected in >3000 MVA-BN recipients at the time of initiation of POX-MVA-013. It is possible that additional instances of troponin I > ULN or clinically significant abnormal ECG may have been missed after the second dose.*

Blood collection for antibody analysis via validated vaccinia specific PRNT GMTs and vaccine specific ELISA GMTs occurred at baseline and then again at week 6 as the primary efficacy endpoint.

An independent Data Safety Monitoring Board reviewed all SAEs and any grade 3 or higher systemic or lab toxicity with a possible causal relationship to the study vaccine in subjects prior to further enrollment or dosing of study vaccine. No events triggered trial halting rules during this study.

6.3.8 Endpoints and Criteria for Study Success

The primary endpoint was PRNT GMTs measured at two weeks after the last MVA-BN vaccination.

All lab specimens for PRNT titer assay were sent to (b) (4), where a validated PRNT assay using VV-WR as reporter virus was performed.

Reviewer Comment: *There are concerns by the assay review team and CMC reviewers about the validity of the LLOQ for PRNT assays used in this and other studies submitted with this BLA. In response to Information Request #13 ("Clinical Information Amendment Response to IR 13" 13 February 2019), it was noted that the version of PRNT assay used in this study was version 3, which had a LLOD of* (b) (4) *and LLOQ of 20; these parameters support the validity of PRNT GMTs reported in this study. In a separate Information Request #32, CBER asked the applicant to impute PRNT GMT that was <LLOQ to 1/2 LLOQ (i.e., 10 in this case) and recalculate PRNT GMTs and seroconversion rates using this approach. The revised data were submitted to STN125678/0.50 on 23 August 2019, and the results presented in this review have reflected the revised data.*

Secondary immunogenicity endpoints for this study included:

- GMTs for validated vaccinia specific ELISA assays obtained at week 6
- Rates of seroconversion via PRNT and ELISA at week 6
- Pearson Correlation Coefficient between $\log_{10}$ transformed PRNT and ELISA titers at week 6.

Seroconversion was defined as either the appearance of antibody titers above the assay LLOD in subjects who were seronegative at baseline or a two-fold or greater increase in antibody titers above baseline in subjects who had pre-existing antibody at baseline.

Reviewer Comment: *The significance of a two-fold rise in anti-vaccinia antibody titers is unclear. In most vaccine clinical trials, seroconversion is defined as a four-fold rise in antibody titer. Given that the rates of baseline seropositivity were low (as would be expected given the eradication of smallpox disease and relative infrequency of other pox viral infections in humans in the United States), between 0.6-1.4% of each study group for ELISA, and were highest in the placebo group, this definition appears reasonable.*

Secondary safety and reactogenicity endpoints were:

- occurrence, relationship and intensity of any SAEs at any point in the study
- cardiac signs/symptoms indicative of myopericarditis (AESIs) at any point in the study
- occurrence of any vaccine-related Grade 3 or 4 AEs within 28 days of vaccination with study vaccine
- occurrence, relationship and intensity of unsolicited non-serious AEs within 28 days of study vaccination
- occurrence, relationship, intensity and duration of solicited local and systemic AEs during the 8-day period after each vaccination.

Severity definitions for local solicited AEs were outlined on the basis of size of erythema, swelling or induration (0, <30mm, 30-100mm or > 100mm) and all were considered related to the vaccine. Severity of generalized solicited AEs were based on level of interference with daily activity and specific thresholds for body temperature (<37.5^{o}C, 37.5-38^{o}C, 38-39^{o}C, 39-40^{o}C and > 40^{o}C). Grading of unsolicited AEs was standardized per study protocol, as were criteria for vaccine relatedness.

Reviewer Comment: *AESIs were defined as new cardiac signs or symptoms, clinically significant ECG changes and elevations of troponin I > 2x ULN, which is considered a Grade 2 troponin elevation per the toxicity grading scale used in this study. Grade 1 elevations of troponin I were not initially reported as AESIs in this study. An information request was sent to the sponsor on 21 December 2018 asking for reporting of Grade 1 elevations of troponin I as AESIs. New rates of troponin elevations, defined as greater than ULN were sent as a Clinical Information Amendment ("Response to FDA Request for Information #8") are now included in this report.*

6.3.9 Statistical Considerations & Statistical Analysis Plan

The primary hypothesis of this study was that the humoral immune responses from the PPS, as assessed by PRNT GMT, at 2 weeks after the 2nd vaccination with MVA-BN from three consecutive lots would be statistically equivalent. The null hypothesis was that there would be a significant variation of mean GMTs between consecutive vaccine lots. The margin of equivalent was pre-set at 0.301 on log10 PRNT titers (equivalent to a factor 2 for PRNT GMT). if the 95% CI for the ratio of GMTs between groups remained between 0.5 and 2 (based on a PRNT delta factor of 2). Based on a previously demonstrated standard deviation of log10 titers for PRNT of 0.85 and assuming that log10 PRNT values would be normally distributed and a significance level of 5% with 80% power, minimum sample size for analysis was calculated to be 600 subjects per group. Additional subjects (for a total of ~1000 per group) were ultimately enrolled to account for potential attrition of 15% and to have a robust safety database.

Seropositivity rates, seroconversion rates, PRNT and ELISA GMTs for each group, correlation of PRNT and ELISA GMT and safety data rates (AEs, SAEs, AESIs) were all calculated on the PPS and FAS and were all analyzed with descriptive methods.

Please refer to the CBER Statistician's review for full details about the statistical analysis plan for this study.

6.3.10 Study Population and Disposition

6.3.10.1 Populations Enrolled/Analyzed

There were three different pre-defined populations in this trial:

- Full Analysis Set (FAS): Included all randomized subjects who received at least 1 dose of study vaccine and for whom any data was available; this set also represented the population on which safety analysis was performed.

- Immunogenicity Analysis Set (IAS): Included the first 700 subjects randomized to each treatment group; secondary immunogenicity analyses were performed on this set.

Reviewer Comment: *For practical reasons, the IAS actually consisted of all subjects enrolled through a certain date (9 Jul 2013), which resulted in the inclusion of slightly more than 700 subjects per group (2,829 total).*

- Per Protocol Set (PPS): Included all subjects from the IAS who received both doses of vaccine/placebo, completed visits 1, 3 and 4 and adhered to all protocol conditions; subjects with minor protocol violations or who did not have visit 2 or 5 within the pre-specified window were still included in this set; primary immunogenicity analyses were performed on this set.

6.3.10.1.1 Demographics

Table 40: Demographic Characteristic of the Full Analysis Set in POX-MVA-013

Variable	Group 1 (N=999)	Group 2 (N=1005)	Group 3 (N=999)	Combined Group 1-3 (N=3003)	Group 4 (N=1002)	All Subjects (N=4005)
Mean Age (SD)	27.6 (6.28)	27.5 (6.24)	28.0 (6.31)	27.7 (6.28)	27.7 (6.38)	27.7 (6.30)
Female Gender (n[%])	526 (52.7)	527 (52.4)	494 (49.4)	1547 (51.5)	539 (53.8)	2086 (52.1)
American Indian/ Alaska Native (n[%])	5 (0.5)	4 (0.4)	4 (0.4)	14 (0.4)	7 (0.7)	20 (0.5)
Asian (n[%])	24 (2.4)	17 (1.7)	18 (1.8)	59 (2.0)	19 (1.9)	78 (1.9)
Black/ African American (n[%])	172 (17.2)	165 (16.4)	191 (19.1)	528 (17.6)	184 (18.4)	712 (17.8)

Variable	Group 1 (N=999)	Group 2 (N=1005)	Group 3 (N=999)	Combined Group 1-3 (N=3003)	Group 4 (N=1002)	All Subjects (N=4005)
Native Hawaiian/ Other Pacific Islander (n[%])	2 (0.2)	7 (0.7)	4 (0.4)	13 (0.4)	3 (0.3)	16 (0.4)
Whie/ Caucasian (n[%])	773 (77.4)	790 (78.6)	765 (76.6)	2328 (77.5)	773 (77.1)	3101 (77.4)
"Other" (n[%])	23 (2.3)	22 (2.2)	17 (1.7)	62 (2.1)	16 (1.6)	78 (1.9)
Hispanic or Latino Ethnicity (n[%])	119 (11.9)	119 (11.8)	108 (10.8)	346 (11.5)	109 (10.9)	455 (11.4)

Source: Original BLA 125678/0; Adapted from POX-MVA-013 Clinical Study Report, p73

Reviewer Comment: *The demographics of each treatment group were similar and generally representative of the population of the United States (proposed population for use). The average age is 27.7 years (range 27.5-28.0) across all groups. There was an even balance between sexes and the proportion of female subjects was equal across groups, between 49.4-52.7%. The average weight for each group was between 76.32-77.39 kg and average BMI was between 26.18 and 26.36 (data not included in this table). The majority of study subjects identified as White/Caucasian (76.6-78.6%), followed by Black/African American (16.4-19.1%), Asian (1.7-2.4%) and Other (1.7-2.3%) which was equal across groups. There were no statistically significant differences between groups.*

6.3.10.1.2 Medical/Behavioral Characterization of the Enrolled Population

Given the risk of pericarditis and myocarditis which has previously been observed with smallpox vaccines, the study population was screened for risk of future cardiovascular disease. Cardiac risk, based on the National Cholesterol Education Program's screening tool [13], family history of ischemic heart disease before the age of 50 and medical history of cardiac disease were considered. Subjects were excluded from enrollment if their "cardiac risk", or risk of myocardial infarction or death within the next 10 years was > 10% and if they had history of any cardiac condition under the care of a physician.

Cardiac risk- Potential subjects were screened for increased cardiac risk (assessing age, gender, race, total cholesterol, HDL, SBP, DBP, diabetes, smoking status) due to previously demonstrated risk of myocarditis and adverse cardiac events with smallpox vaccines. Subjects were excluded if they had > 10% increased risk and the remainder were stratified by risk level. Overall, most subjects had 0-1% risk of cardiac disease. Less than 10% of study population had a cardiac risk > 1%. Out of 3634 randomized subjects who had data about cardiac risk, a slightly higher percentage of subjects had increased cardiac risk (2-9% vs 0-1%) in Group 3 (n = 91, 9.9%) versus the other groups (Group 1 7.3% (n=66), Group 2 7.8% (n=71), Placebo 7.99% (n=73)).

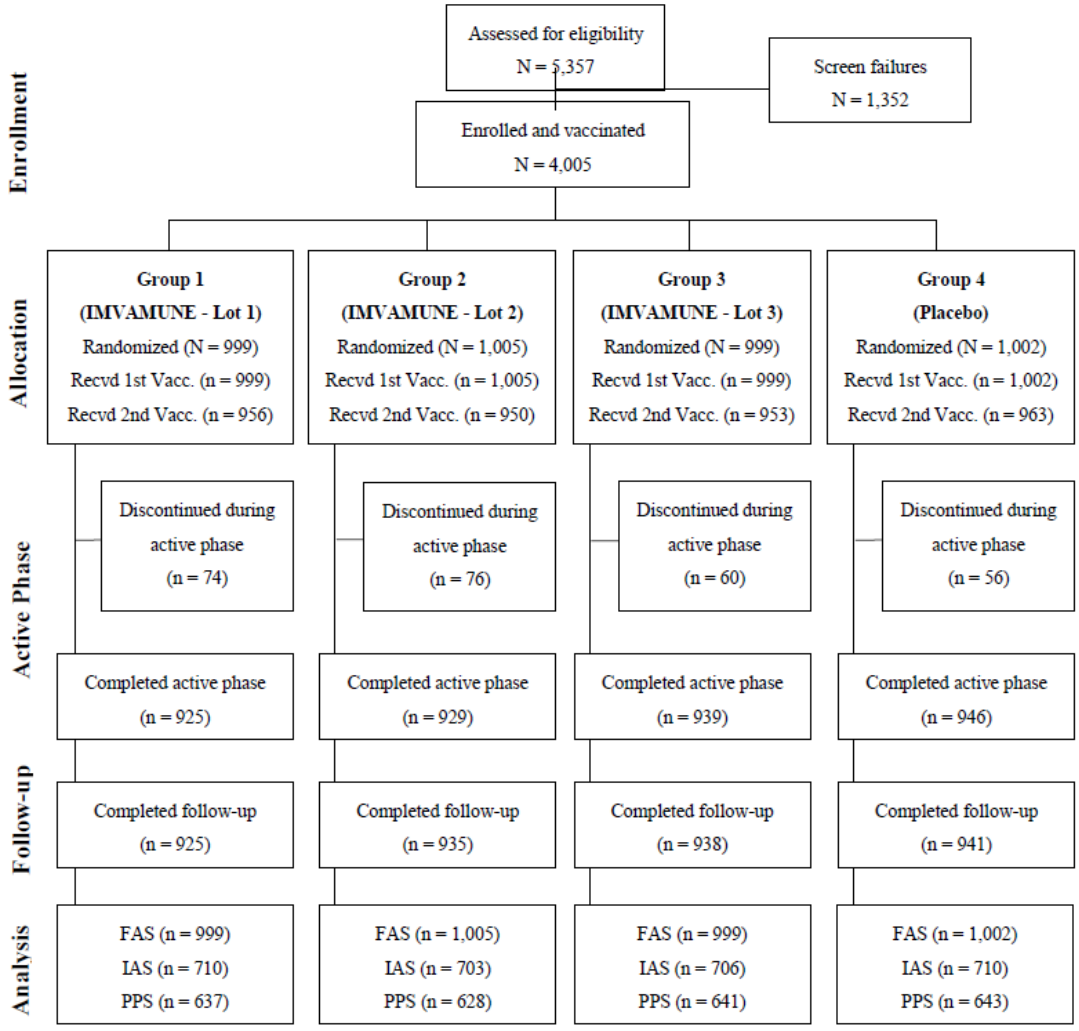
Reviewer Comment: *The difference in low level cardiac risk between groups is most likely not clinically significant. Of note, there are now more recent cardiac risk screening tools (American College of Cardiology/American Heart Association*

in 2013), however these were published after the initial study design and planning phases of this study.

6.3.10.1.3 Subject Disposition

Overall, 5357 individuals were screened for eligibility, 1352 individuals were excluded from the study as screen failures and 4005 subjects were enrolled and vaccinated. Subjects were randomized evenly across the four study groups (N= 999, 1005, 999, 1002 in Groups 1, 2, 3 and 4 respectively). A higher number of subjects were discontinued during the active phase of the study in Groups 1 and 2 (N=74 and N=76) compared to Groups 3 and 4 (N=60 and N=56). Ultimately, similar numbers of subjects in each group were included in the Per Protocol Set for primary immunogenicity analysis; N=637, 628, 641 and 643 for Groups 1-4 respectively (Fig 4).

Figure 4: Subject Disposition in POX-MVA-013



FAS = Full Analysis Set; FU = Follow-Up; IAS = Immunogenicity Analysis Set, subset used for immunogenicity analysis (first 700 subjects enrolled per group, total of subjects =2,829); PPS = Per Protocol Set; n = Number of subjects in the specified category.

Source: Original BLA 125678/0; Clinical Study Report POX-MVA-013, p. 68

Reviewer Comment: *Rates of discontinuation and rates of study completion were equal across study groups. The majority of protocol violations, which resulted in exclusion from the PPS, were due to antibody sampling errors (n=63), failure to adhere to visit schedule (n=53) and prolonged immunogenicity sample turn-around-time (n=31*). *Site 110 had the highest proportion of subjects excluded from the study for major protocol deviations. Only 13 subjects total (out of 31 subjects who were randomized at Site 110, or 41.9%) did not have a major protocol deviation. Sixteen (16) subjects had delayed immunogenicity sample turn-around time, which was considered a major protocol deviation. Other major deviations included failure to adhere to visit schedule (1) and antibody sample processing/data recording error (1). Exclusions for major deviations were evenly distributed across treatment groups at this site. It does not appear that any subjects from this site were included in the PPS due to the enrollment-date-based inclusion of subjects into the PPS. The sponsor provided adequate explanation for these violations and exclusion from the PPS in "Response to FDA Request for Information #13 (Feb 12, 2019)."*

6.3.11 Efficacy Analyses

6.3.11.1 Analyses of Primary Endpoint

The primary endpoint of this study was the equivalence of GMTs of vaccinia specific PRNT assays after two MVA-BN vaccinations from one of three consecutive lots, which was obtained at trial visit 4 (Week 6). Primary immunogenicity analyses were performed on the IAS and the PPS.

As shown in Table 41, for the PPS (n= 2549), all groups had a baseline PRNT GMT of 1.0, indicating no previous smallpox or vaccinia exposure. All 3 treatment groups demonstrated a similar rise in GMT at 2 weeks after the second dose of vaccine (Week 6). Subjects in the placebo group continued to have a GMT of 1.0 at Week 6.

Table 41: Per Protocol Set PRNT GMTs at All Sampling Points in POX-MVA-013

Group (N for PPS)	Visit 1 (Day 0) GMT	Visit 1 (Day 0) 95% CI	Visit 4 (Day 42) GMT	Visit 4 (Day 42) 95% CI
Group 1 (637)	10.1	10.0,10.2	110.5	103.3, 118.1
Group 2 (628)	10.0	10.0,10.1	100.7	94.0, 107.9
Group 3 (641)	10.1	10.0,10.3	117.0	108.9, 125.8
Group 4 (643)	10.1	10.0,10.2	10.1	10.0,10.3

Source: Original BLA 125678/0.50; Response to Request for Information #32, Table 6, p 9

The ratio of PRNT GMTs were compared between treatment groups and ranged between 0.8605 (Group 2 to 3) and 1.0970 (Group 1 to 2). These ratios fell within the pre-defined range for equivalence of 0.5-2.0, so equivalent PRNT Ab responses were demonstrated across the 3 consecutively produced vaccine lots that were used in this study (Table 42).

Table 42: Per Protocol Set PRNT Visit 4 GMT Equivalence Analysis

Groups Compared	GMT Ratio	95% CI	Equivalence Met (Y/N)
Group 1/ Group 2	1.0970	0.9967,1.2075	Y
Group 1/ Group 3	0.9440	0.8554, 1.0418	Y
Group 2/ Group 3	0.8605	0.7788, 0.9508	Y

Source: Original BLA 125678/0.50; Response to Request for Information #32, Table 7, p. 9

Reviewer Comment: *There was a statistically significant difference between Group 2 and Group 3 GMTs, in that they had non-overlapping 95% confidence intervals, however this difference was within the acceptable range per pre-specified criteria.*

Results were similar when equivalence analysis was performed on IAS (n=2829). Baseline (Visit 1) PRNT GMTs for Groups 1-4 were all 1.0 (95% CI 1.0-1.1). PRNT GMTs at week 6 were higher, 109.2 (95% CI 102.1-119.9), 101.8 (95.1-109.1), 116.2 (108.2-124.8), for Groups 1-3 respectively and remained 1.0 (1.0 - 1.1) for Group 4. Similar to the PPS, all three vaccine lots were considered equivalent per pre-defined immunogenicity equivalence parameters, with ratios of 1.0727 (0.9741-1.1812 95% CI) for Group 1/Group 2, 0.9400 (0.8520-1.0371) for Group 1/Group 3 and 0.8763 (0.7938-0.9673) for Group 2/Group 3.

6.3.11.2 Analyses of Secondary Endpoints

Secondary immunogenicity endpoints for POX-MVA-013 included:

- vaccinia specific ELISA GMTs
- correlation of PRNT and ELISA titers
- rates of seropositivity and seroconversion for both PRNT and ELISA across study groups receiving the vaccine.

Vaccinia specific ELISA GMTs across the 3 vaccine study groups were equivalent (ratios 0.8392-1.1342), similar to PRNT titers. Baseline ELISA GMTs in all groups were slightly higher on average (1.1-1.3) compared to PRNT but with a corresponding higher rise by week 6 in vaccine groups (Group 1- 901.0, Group 2- 794.4, Group 3- 946.7) with no change in placebo GMT (Group 4-1.2).

PRNT seropositivity was low across all groups at baseline (0.3-0.6%) with a rise to 97.0 - 97.7% seropositivity in all subjects who received two doses of vaccine (95%CI 96.7-98.4)), with no rise demonstrated in the placebo group. Rates of ELISA seropositivity were slightly higher at baseline (2.9-6.4%) than for PRNT but demonstrated a similar rise after two doses of vaccine (99.5-100%) with no rise in placebo ELISA seropositivity (4.7% at baseline to 4.2% at week 6).

Seroconversion rates for both PRNT and ELISA were > 96% (96.8-97.7% PRNT, 99.5-100% ELISA) for all study vaccine groups and remained 0.2-2.1% (PRNT and ELISA respectively) for placebo for both the PPS and IAS.

Statistically significant correlation (p<0.0001) between PRNT and ELISA GMTs, with Pearson correlation coefficients (r) of 0.572-0.631 for Groups 1-3 and 0.447 for Group 4/Placebo was noted.

6.3.11.3 Subpopulation Analyses

Analysis of PRNT titers from Visit 1 (Baseline) and Visit 4/Week 6 for the IAS stratified by gender did not demonstrate any significant differences between male and female subjects in each treatment group.

Analysis of PRNT titers from Visit 1 and Visit 4 for the IAS, stratified by race, demonstrated lower mean Visit 4 titers in Black/African American subjects across all groups which received the vaccine (Groups 1-3) when compared to titers in the White/Caucasian subjects and Asian group subjects. This is most notable in Group 3, which generally had a higher mean Visit 4 PRNT titers across study groups (White-192.16, Asian-246.11, Other 178.9) except in Black/African American subjects (129.39). When stratified by race (including Black/African-American subjects), equivalence criteria (a factor difference in the log10 titers <2) were still met, supporting the primary study objective of confirming lot consistency.

Reviewer Comment: *Though a trend exists for lower PRNT and ELISA titers in Black/African-American subjects, the percentages of subjects seroconverted (secondary study endpoint) is similar between racial groups. In the pivotal study to demonstrate vaccine effectiveness, POX-MVA-006, the MVA-BN peak visit GMT for Black/African-American subjects was higher than the peak visit GMT for the overall study population and met the pre-specified non-inferiority criterion compared to the ACAM2000 peak visit GMT for Black/African-American subjects.*

Of note, one subject (POX-MVA-(b) (6) *), a 40-year-old Asian male in Group 3, had a significantly elevated ELISA (50) and PRNT titers at baseline (143) with a very robust post-vaccine response (ELISA- 812, PRNT 601). These values are dramatic outliers and raise the question of possible previous vaccinia or other Poxviridae exposure in this subject. Alternatively, it is possible these results were erroneous due to assay or specimen processing issues. These subpopulation analyses do not reflect re-calculated PRNT GMTs with values below the LLOQ imputed to 10 (1/2 of the LLOQ), however as the values were not significantly changed in the overall population and there were not major differences between subgroups with the initial analysis, it is not expected that the subgroup population analyses would be significantly different as a result of the recalculation.*

6.3.11.4 Dropouts and/or Discontinuations

Overall, 266 subjects (6.6% FAS, 10.4% PPS) did not complete the study (i.e. dropped out or were discontinued prior to completion of follow up at Visit 5). The percentages of subjects who were discontinued during the active phase of this study were evenly distributed between study groups, ranging from 5.6% (n=56) in Group 4 to 7.6% (n=76) in Group 2. Eleven of these subjects (0.2%) were

withdrawn due to adverse events. The low numbers of discontinued subjects (<20%) would not be expected to have a significant impact on the outcomes of this study. Additionally, a very small number of subjects were excluded from analysis following unblinding of the study data due to major protocol deviations (n= 13, 0.3% of overall FAS, up to 2% of PPS groups) which would potentially introduce bias of the data, however, again, this number is low and should not significantly impact overall results.

Immunogenicity analysis of only subjects who received both doses of vaccine is appropriate. This is a reasonable approach, given that peak antibody response to MVA-BN vaccine is known to occur after the 2nd dose.

6.3.12 Safety Analyses

6.3.12.1 Methods

All randomized subjects who received at least 1 vaccination (FAS, n= 4,005) were included in the safety analysis. Both solicited and unsolicited adverse events were collected in this study.

Local and systemic adverse events following vaccination were collected via a memory aid for the first 8 days (Day 0-7) after each dose of study vaccination. Solicited local AEs included:

- erythema
- swelling
- induration
- pruritus
- pain of the injection site.

Unsolicited AEs were inquired about and documented at active trial visits (Visit 1 to Visit 5) and until resolution if ongoing at Visit 5. Grade 3 or 4 abnormal laboratory values, collected at Visit 2 and 4 routinely (also as needed for new symptoms or AEs), were also documented as AEs.

SAEs and AESIs were documented at all trial visits, including the Week 30 follow up visit. As a part of screening for cardiac risk prior to enrollment, all subjects had an ECG and Troponin I level at screening and those enrolled had repeat ECG and Troponin at Visit 2 (2 weeks after the first vaccination).

6.3.12.2 Overview of Adverse Events

Overall, 90.9% (n=2,731) of subjects who received a dose of MVA-BN (Groups 1-3) reported an adverse event, compared to 60% (n=601) of subjects who received placebo vaccination. AE rates were similar amongst vaccine groups (91.8% vs 90.1% vs 90.9% in Groups 1, 2 and 3 respectively). Rates of SAEs were equal between treatment and placebo groups, with 25 (0.8%) subjects reporting at least one SAE in the combined treatment groups and 8 subjects

(0.8%) in the Placebo group reporting at least one SAE. There was a higher rate of treatment emergent AESIs in the combined vaccine groups (n=8, 0.3%) than in placebo (n=1, 0.1%). There was one death in this trial, in Group 1, which was due to suicide and considered not related to the study vaccine. Please refer to Table 43 for an overview of adverse events in this study.

Table 43: Overview of Solicited and Unsolicited Adverse Events (Full Analysis Set)

Number of Subjects with At Least 1:	Group 1 (Lot 1) (N = 999) n (%)	Group 2 (Lot 2) (N = 1005) n (%)	Group 3 (Lot 3) (N = 999) n (%)	Combined Groups 1-3 (All MVA-BN) (N=3003) n (%)	Group 4 (Placebo) (N = 1002) n (%)	Fisher's Exact Test (p-value) (Groups 1-3 vs 4) n (%)
AE	917 (91.8)	906 (90.1)	908 (90.9)	2731 (90.9)	601 (60.0)	< 0.0001
TEAE	913 (91.4)	903 (89.9)	900 (90.1)	2716 (90.4)	581 (58.0)	<0.0001
SAE	11 (1.1)	7 (0.7)	7 (0.7)	25 (0.8)	8 (0.8)	>0.9999
TE AESI	2 (0.2)	5 (0.5)	1 (0.1)	8 (0.3)	1 (0.1)	0.4654
Related TEAE	565 (56.6)	559 (55.6)	576 (57.7)	1700 (56.6)	349 (34.8)	NC
Related SAE	0	0	0	0	1 (0.1)	NC
TEAE Grade ≥ 3	155 (15.5)	142 (14.1)	132 (13.2)	429 (14.3)	53 (5.3)	NC
Related TEAE Grade ≥ 3	58 (5.8)	57 (5.7)	53 (5.3)	168 (5.6)	27 (2.7)	NC
Related TE AESI	0	1 (0.1)	1 (0.1)	2 (0.1)	0	NC
AE Leading to Withdrawal from Trial	5 (0.5)	9 (0.9)	4 (0.4)	18 (0.6)	3 (0.3)	NC
AE Leading to Withdrawal from Vaccination	6 (0.6)	8 (0.8)	5 (0.5)	19 (0.6)	3 (0.3)	NC
Deaths	1 (0.1)	0	0	1 (0.0)	0	NC

Source: Original BLA 125678/0; Adapted from POX-MVA-013 Clinical Study Report, p.083
N=total number of subjects in the specified group, n=number of subjects with specified events

Solicited Adverse Reactions

More subjects who received MVA-BN in this study reported injection site pain (84.3-85.9%) compared to those who received placebo (19.1%) (Table 44). Of those subjects, 6.8-7.7% graded their pain as Grade 3 (compared to 1.0% in placebo). Erythema (59.2-62.5%), swelling (50.8-52.6%), induration (45.0-46.1%) and pruritus (42.2-43.7%) of the injection site were also reported equally across all vaccine groups; however, Grade 3 erythema, swelling, induration and pruritus were reported in less than two percent of subjects in all groups. Rates of local solicited adverse events of any severity were similar after the first and second

vaccine for all groups, other than a lower percentage of subjects reporting injection site pain after the second injection in all groups (Vaccination 1- 79.3% vs Vaccination 2- 69.5%, 75.9% vs 66.6%, 76.9% vs 66.4%, 14.3% vs 10.1% in Groups 1-4 respectively). Rates of severe (Grade 3 or higher) local solicited adverse events were similar following the first versus second vaccination, other than a slight increase in Grade 3 erythema (1.5% versus 0.1%) after vaccination 2. Average duration of local AEs was longer following the first vaccine (IQR 3-9 days) versus the second (IQR 2-5 days) for all vaccine groups.

A higher percentage of subjects who received MVA-BN (59.5%) had at least one solicited general adverse event compared to Placebo (38.9%) (Table 44). The most common AEs experienced by subjects after receiving MVA-BN were myalgia (42.8%), headache (34.8%), fatigue (30.4%), nausea (17.3%) and chills (10.4%). Mean duration of these general AEs was 2 to 5 days. Most of these AEs were mild or moderate, with the percentage of solicited general AEs rated as severe (Grade 3 or higher) ranging from 1.0% of chills (0.7-1.2%) to 3.0% of fatigue (2.8-3.2%). One-quarter to one-half of solicited myalgia (40.7%), headaches (31.0%) and fatigue (27.9%) were considered related to MVA-BN. This compares to the most common general AEs experienced by the Placebo group, which were headache (25.6%), fatigue (20.5%), myalgia (17.6%) and nausea (13.1%). Mean duration was slightly shorter (2-3 days) and severity was lower overall, with the percentage of AEs graded as severe ranging from 0.3% of chills to 2.1% of headache. General AEs of all types were more common during the first vaccination period than the second (1014 vs 716 for Group 1; 985 vs 768 for Group 2; 1019 vs 710 for Group 3; 641 vs 345 for Group 4) with the exception of pyrexia (0.8% in combined groups 1-3 post vaccination 1 vs 1.1% post vaccination 2).

Pyrexia was present in less than 2% of all subjects, regardless of treatment group and severe pyrexia (Grade ≥3) occurred in less than 0.5% (n=5) of subjects who received MVA-BN (compared to 0 in Placebo group).

Table 44: Overview of Solicited Local and Systemic Adverse Reactions, Total and Grade ≥ 3 (Full Analysis Set)

Solicited Adverse Reaction	MVA-BN (N=2943), n (%)	Placebo (N=980), n (%)
Injection Site Pain	2499 (84.9)	187 (19.1)
Pain Grade ≥3	218 (7.4)	10 (1.0)
Injection Site Erythema	1788 (60.8)	173 (17.7)
Erythema Grade ≥3	45 (1.5)	0
Injection Site Swelling	1520 (51.6)	55 (5.6)
Swelling Grade ≥3	23 (0.8)	0
Injection Site Induration	1335 (45.4)	45 (4.6)
Induration Grade ≥3	10 (0.3)	0
Injection Site Pruritus	1268 (43.1)	115 (11.7)
Pruritus Grade ≥3	48 (1.6)	2 (0.2)
Pyrexia	50 (1.7)	9 (0.9)
Pyrexia Grade ≥3	7 (0.2)	0
Headache	1024 (34.8)	251 (25.6)
Headache Grade ≥3	72 (2.4)	21 (2.1)
Myalgia	1259 (42.8)	172 (17.6)
Myalgia Grade ≥3	77 (2.6)	7 (0.7)
Chills	306 (10.4)	57 (5.8)
Chills Grade ≥3	29 (1.0)	3 (0.3)
Nausea	508 (17.3)	128 (13.1)
Nausea Grade ≥3	45 (1.5)	12 (1.2)
Fatigue	895 (30.4)	201 (20.5)
Fatigue Grade ≥3	88 (3.0)	13 (1.3)

Source: Original BLA 125678/0; Adapted from POX-MVA-013 Clinical Study Report, Tables 19 and 20, p. 84-87
N=total number of subjects in the specified group, n=number of subjects with specified events

Unsolicited Treatment Emergent Adverse Events (TEAEs)

Overall, 660 (22%) subjects who received MVA-BN (Groups 1-3 combined) reported a total of 975 unsolicited TEAEs, compared to 189 (18.9%) subjects reporting 283 TEAEs in the Placebo group (Table 45). Most TEAEs (600 [61.5%] in MVA-BN and 158 [55.8%] in placebo) occurred following the first vaccination. Subjects most commonly reported 1 or more TEAEs in the following System Organ Classes (SOC): Infections and Infestations (n= 304 subjects; Groups 1-3: 234 subjects [7.8%], Group 4: 70 subjects [7.0%]), General and Administration Site Disorders (n= 149 subjects, Groups 1-3: 136 [3.9%], Group 4: 13 [1.0%]), Respiratory, Thoracic and Mediastinal Conditions (n=145 subjects, Groups 1-3: 75 [2.5%], Group 4: 16 [1.6%]), Injury, Poisoning and Procedural Complications (n=91 subjects, Groups 1-3: 67 [2.2%), Group 4: 24 [2.4%]) and Gastrointestinal Disorders (n=88 subjects , Groups 1-3: 67 [2.2%], Group 4: 21[2.1%]).

Additionally, 2.1% (n=62) of subjects who received MVA-BN experienced AEs under Nervous System Disorders SOC, which is higher than in placebo (n=13, 1.3%). This difference is mostly accounted for due to increased reports of headache (Groups 1-3: n=22, 0.7% vs Group 4: n=5, 0.5%) and dizziness (Groups 1-3: n=15, 0.5% vs Group 4: n=2, 0.2%) in the MVA-BN groups.

Table 45: Treatment Emergent Unsolicited Adverse Event by System Organ Class (Full Analysis Set) in POX-MVA-013

System Organ Class	Group 1 (Lot 1) (N=999) n (%)	Group 2 (Lot 2) (N=1005) n (%)	Group 3 (Lot 3) (N= 999) n (%)	Combined Groups 1-3 (N=3003) n (%)	Group 4 (Placebo) (N=1002) n (%)
At least 1 TEAE	199 (19.9)	238 (23.7)	223 (22.3)	660 (22.0)	189 (18.9)
Infections and Infestations	61 (6.1)	90 (9.0)	83 (8.3)	234 (7.8)	70 (7.0)
General Disorders and Administration Site Conditions	34 (3.4)	41 (4.1)	43 (4.3)	118 (3.9)	10 (1.0)
Respiratory, Thoracic and Mediastinal Disorders	27 (2.7)	27 (2.7)	21 (2.1)	75 (2.5)	16 (1.6)
Gastrointestinal Disorders	18 (1.8)	25 (2.5)	24 (2.4)	67 (2.2)	21 (2.1)
Injury, Poisoning and Procedural Complications	21 (2.1)	27 (2.7)	19 (1.9)	67 (2.2)	24 (2.4)
Nervous System Disorders	23 (2.3)	21 (2.1)	18 (1.8)	62 (2.1)	13 (1.3)
Skin and Subcutaneous Tissue Disorders	17 (1.7)	13 (1.3)	22. (2.2)	52 (1.7)	17 (1.7)
Musculoskeletal and Connective Tissue Disorders	14 (1.4)	16 (1.6)	16 (1.6)	46 (1.5)	22 (2.2)
Investigations	9 (0.9)	8 (0.8)	13 (1.3)	30 (1.0)	15 (1.5)
Psychiatric Disorders	4 (0.4)	10 (1.0)	3 (0.3)	17 (0.6)	8 (0.8)
Blood and Lymphatic Disorders	3 (0.3)	4 (0.4)	6 (0.6)	13 (0.4)	2 (0.2)
Reproductive System and Breast Disorders	2 (0.2)	6 (0.6)	3 (0.3)	11 (0.4)	5 (0.5)
Vascular Disorders	4 (0.4)	5 (0.5)	2 (0.2)	11 (0.4)	0
Cardiac Disorders	2 (0.2)	4 (0.4)	3 (0.3)	9 (0.3)	1 (0.1)
Eye Disorders	3 (0.3)	4 (0.4)	2 (0.2)	9 (0.3)	2 (0.2)
Renal and Urinary Disorders	2 (0.2)	3 (0.3)	2 (0.2)	7 (0.2)	4 (0.4)
Surgical and Medical Procedures	2 (0.2)	2 (0.2)	2 (0.2)	6 (0.2)	2 (0.2)
Ear and Labyrinth Disorders	2 (0.2)	0	2 (0.2)	4 (0.1)	2 (0.2)
Metabolism and Nutrition Disorders	1 (0.1)	1 (0.1)	2 (0.1)	4 (0.1)	2 (0.2)
Immune System Disorders	3 (0.3)	0	0	3 (0.1)	3 (0.3)
Congenital, Familial and Genetic Disorders	0	1 (0.1)	1 (0.1)	2 (0.1)	0
Hepatobiliary Disorders	1 (0.1)	1 (0.1)	0	2 (0.1)	2 (0.2)
Endocrine Disorders	0	1 (0.1)	0	1 (0.0)	0

Source: Original BLA 125678/0; Adapted from POX-MVA-013 Clinical Study Report, Table 21, p 88-89.
N=total number of subjects in the specified group, n=number of subjects with specified events

The most commonly reported unsolicited specific TEAEs (by Preferred Term) in subjects receiving MVA-BN were upper respiratory tract infection (n=58, 1.9%), injection site induration (n=50, 1.7%), nasopharyngitis (n=36, 1.2%), and injection site hematoma (n=24, 0.8%).

These findings compare to higher rates of upper respiratory tract infection (n= 31, 3.1%) in the placebo group, but lower rates of injection site hematoma (n=5, 0.5%), nasopharyngitis (n=3, 0.3%) and no reports of injection site induration.

TEAEs by Intensity/Severity and Relationship to Study Vaccine

Of the 975 unsolicited TEAEs experienced by subjects who received MVA-BN, 926 (95.0%) were mild or moderate in severity (Grade 1-2) (Table 46). Conversely, forty-nine (5.0%) were considered Grade 3 or higher (four were Grade 4). Compared to TEAEs in the placebo group: 275 (97.2%) were mild or moderate and 2.8% were Grade 3 or higher. There was a slightly higher proportion of Grade 3 or higher TEAEs following the second vaccination period than in the first vaccination period (5.1% vs 4.3% in MVA-BN, 3.2% vs 2.5% in Placebo).

Table 46: Treatment Emergent Unsolicited Adverse Events (Per Vaccination Period) By Intensity in POX-MVA-013

Intensity	MVA-BN (Vaccination Period 1)	MVA-BN (Vaccination Period 2)	Placebo (Vaccination Period 1)	Placebo (Vaccination Period 2)
Grade 1	424 (70.7%)	220 (58.7%)	104 (65.8%)	88 (70.4%)
Grade 2	148 (24.7%)	134 (35.7%)	50 (31.6%)	33 (26.4%)
Grade 3	26 (4.3%)	19 (5.1%)	4 (2.5%)	4 (3.2%)
Grade 4	2 (0.3%)	2 (0.5%)	0	0
Total	600	375	158	125

Source: Original BLA 125678/0; Adapted from POX-MVA-013 Clinical Study Report Table 15.3.1.4.9, p 1271-1272

Most TEAEs were considered unrelated to treatment (n= 602, 61.7% MVA-BN; n=191, 67.5% Placebo), and 20.6% (n=201) were considered at least possibly related to MVA-BN vaccine (Table 47). Less than 10% were considered definitely related to treatment (n=90, 9.2% MVA-BN; n=4, 1.4% Placebo) overall. More TEAEs were considered at least possibly related (29.3% vs 16.4%) or definitely related (14.2% vs 1.3%) to study treatment after the first dose of MVA-BN compared to the second. The difference in the number of definitely related TEAEs after the first (n= 85) versus second (n=5) dose of MVA-BN is largely accounted for by unsolicited reports of injection site hematoma (n= 23 after vaccination 1 versus n=2 after vaccination 2) and induration (n= 35 after vaccination 1 versus none after vaccination 2).

Table 47: Treatment Emergent Unsolicited Adverse Events (Per Vaccination Period) by Relationship in POX-MVA-013

Relatedness	MVA-BN (Vaccination Period 1)	MVA-BN (Vaccination Period 2)	Placebo (Vaccination Period 1)	Placebo (Vaccination Period 2)
Unrelated/None	324 (54.0%)	278 (74.1%)	105 (66.5%)	86 (68.8%)
Unlikely	99 (16.5%)	59 (15.7%)	26 (16.5%)	25 (20.0%)
Possible	73 (12.2%)	31 (8.3%)	17 (10.8%)	13 (10.4%)
Probable	17 (2.8%)	1 (0.3%)	6 (3.8%)	0
Definite	85 (14.2%)	5 (1.3%)	3 (1.9%)	1 (0.8%)
Missing	2 (0.3%)	1 (0.3%)	1 (0.6%)	0
Total	600	375	158	125

Source: Original BLA 125678/0; Adapted from POX-MVA-013 Clinical Study Report Table 15.3.1.4.9, p 1271-1272

Of related TEAEs (including Possible, Probable or Definite related events), most were Grade 1 (78.6%) or Grade 2 (17.2%) for MVA-BN recipients (Table 48). Nine (4.2%) related treatment emergent adverse events were graded as severe which included: upper respiratory tract, sinusitis and migraine in Group 1; nausea, vomiting, dizziness and influenza-like illness in Group 2; migraine and injection site cellulitis in Group 3. Severe related TEAEs were more likely in male subjects (n=6; 66.6%) than female subjects (n=3; 33.3%) and in White (n=8; 88.8%) or Black/African American subjects (n=1;11.1%).

Table 48: Summary of Intensity of Treatment Emergent Unsolicited Adverse Events Related to The Trial Vaccine (Full Analysis Set) in POX-MVA-013

Intensity	Group 1 (N = 999) # Events (%)	Group 2 (N =1005) # Events (%)	Group 3 (N = 999) # Events (%)	Combined Groups 1-3 (N = 3003) # Events (%)	Group 4 (N =1002) # Events (%)
Grade 1	43 (70.5)	60 (78.9)	66 (84.6)	169 (78.6)	28 (68.3)
Grade 2	15 (24.6)	12 (15.8)	10 (12.8)	37 (17.2)	12 (29.3)
Grade 3	3 (4.9)	4 (5.3)	2 (2.6)	9 (4.2)	1 (2.4)
Grade 4	0	0	0	0	0
Total	61 (100)	76 (100)	78 (100)	215 (100)	41 (100)

Source: Original BLA 125678/0. Adapted from POX-MVA-013 Clinical Study Report, p 90.

Reviewer Comment: *Group 3 had the highest percentage of mild related events (84.6%) while Group 1 had the lowest (70.5%), while Group 1 had the highest percentage of moderate related events (24.6%), which was nearly double the proportion in Group 3 (12.8%). Group 2 had the highest percentage of severe related events at more than 5%, three of which (nausea, vomiting and dizziness) were from the same subject ((b) (6)). As related events represent a relatively small proportion of overall adverse events, these findings are likely not clinically significant.*

Treatment Emergent Adverse Events by Sex

Overall, a higher percentage of women [n=386 (25%) in MVA-BN groups; 112 (20.8%) in placebo] reported TEAEs, compared to men [n=274 (18.8%) in MVA-BN groups, n=77 (16.6%) in placebo] (Table 49). In the MVA-BN treatment groups, there were higher incidences of female subjects with TEAEs, by SOC, in Infections and Infestations (10.3% vs 5.2%) due primarily to increased counts of upper respiratory tract infection (2.5% vs 1.3%), nasopharyngitis (1.5% vs 0.9%), urinary tract infection (1.0% vs 0.1%) sinusitis (0.9% vs 0.2%) combined vaginocervical infections (0.9% vs 0) and gastroenteritis (0.8% vs 0.2%). There were also higher incidences of TEAEs in women who received MVA-BN, compared to their male counterparts, in SOC groups of General Disorders and Administration Site Conditions (4.2% vs 3.6%), Nervous System Disorders (2.7% vs 1.4%), Reproductive System and Breast Disorders (0.6% vs 0.1%) and Skin and Subcutaneous Disorders (2.3% vs 1.2%).

Table 49: Subjects with at least 1 TEAE by System Organ Class, Treatment and Sex in POX-MVA-013

System Organ Class	Female MVA-BN	Male MVA-BN	Female Placebo	Male Placebo
Subjects with ≥1 TEAE	386 (25.0%)	274 (18.8%)	112 (20.8%)	77 (16.6%)
Infections	159 (10.3%)	75 (5.2%)	46 (8.5%)	24 (5.2%)
Gen/Admin	65 (4.2%)	53 (3.6%)	8 (1.5%)	2 (0.4%)
Nervous	41 (2.7%)	21 (1.4%)	7 (1.3%)	6 (1.3%)
Resp/Thoracic	40 (2.6%)	35 (2.4%)	9 (1.7%)	7 (1.5%)
Gastrointestinal	36 (2.3%)	31 (2.1%)	14 (2.6%)	7 (1.5%)
Skin/SubQ	35 (2.3%)	17 (1.2%)	10 (1.9%)	7 (1.5%)
Injury/Poison	32 (2.1%)	35 (2.4%)	10 (1.9%)	14 (3.0%)
MSK/Connective	27 (1.7%)	19 (1.3%)	14 (2.6%)	8 (1.7%)
Investigations	14 (0.9%)	16 (1.1%)	7 (1.3%)	8 (1.7%)
Psych	11 (0.7%)	6 (0.4%)	5 (0.9%)	3 (0.6%)
Repro/Breast	10 (0.6%)	1 (0.1%)	5 (0.9%)	0
Blood/Lymph	8 (0.5%)	5 (0.3%)	1 (0.2%)	1 (0.2%)
Vascular	5 (0.3%)	6 (0.4%)	0	0
Metabolism	4 (0.3%)	0	1 (0.2%)	1 (0.2%)
Eye	4 (0.3%)	5 (0.3%)	1 (0.2%)	1 (0.2%
Ear/Labyrinth	3 (0.2%)	1 (0.1%)	1 (0.2%)	1 (0.2%)
Cardiac	3 (0.2%)	7 (0.4%)	1 (0.2%)	0
Surgical Procedures	3 (0.2%)	3 (0.2%)	1 (0.2%)	1 (0.2%
Hepatobiliary	2 (0.1%)	0	2 (0.4%)	0
Immune	2 (0.1%)	1 (0.1%	1 (0.2%)	2 (0.4%)
Renal	2 (0.1%)	5 (0.3%)	1 (0.2%)	3 (0.6%)
Endocrine	1 (0.1%)	0	0	0
Genetic	0	2 (0.1%)	0	0

Source: Original BLA 125678/0. Adapted from POX-MVA-013 Clinical Study Report Table 15.3.1.4.2.1, p 1083-1113.

Reviewer Comment: *Overall rates of TEAEs, particularly for infectious symptoms, were higher for female subjects across study groups. However, when comparing rates of TEAEs between female subjects who received the vaccine versus placebo, rates of AEs are still higher for women, which may be a factor of*

reporting bias (i.e. women in the study population are more likely overall to report adverse events, or certain adverse events, than men).

Treatment Emergent Adverse Events by Race

By race, the highest percentage of subjects reporting TEAEs were Asian (n= 15, 25.4% in MVA-BN groups and n=7, 36.8% in Placebo or "P" as denoted in Table 50) but in very low numbers overall, as Asian subjects only accounted for 1.9% of the total study population. Asian subjects, both in MVA-BN and Placebo groups, with TEAEs of SOC General Disorders/Administration Site (8.5% and 5.3% respectively) and Infections and Infestations (8.5% and 21.1%) were disproportionately represented.
The lowest rates of TEAEs were reported by American Indian/Alaska Native (AI/AN) subjects, as only 2 TEAEs (dyspnea and dysphagia) were reported by a single subject (7.7% of AI/AN study population).

Not included in the table below are subjects who listed race as "Other", which accounted for 1.9% of the total study population (N=62 MVA-BN groups, n-16 in placebo). Of subjects who racially identified as "Other", 16.1% (n=10) in MVA-BN groups and 12.5% (n=2) in placebo reported TEAEs. The SOC category with the most TEAEs from this group was Infections/Infestations (n=5, 8.1% MVA-BN, n=2, 12.5% Placebo). Two subjects (3.2%) reported TEAEs in Skin and Subcutaneous Tissue Disorders and one subject each (1.6%) reported TEAEs in Blood and Lymphatic System Disorders, Ear and Labyrinth Disorders, Gastrointestinal Disorders, General Disorders and Administration Site Conditions and Injury, Poisoning and Procedural Complications, all from subjects who received MVA-BN.

Table 50: Treatment Emergent Unsolicited Adverse Events by System Organ Class, Treatment and Race in POX-MVA-013

SOC	AI/AN MVA (%)	AI/AN P (%)	Asian MVA (%)	Asian P (%)	Black MVA (%)	Black P (%)	H/PI MVA (%)	H/PI P (%)	White MVA (%)	White P (%)
At least ≥ 1 TEAE	1 (7.7)	0	15 (25.4)	7 (36.8)	90 (17.0)	25 (13.6)	3 (23.1)	1 (33.3)	541 (23.2)	154 (19.9)
Blood/Lymph	0	0	0	0	5 (0.9)	1 (0.5)	0	0	7 (0.3)	1 (0.1)
Cardiac	0	0	0	1 (5.3)	2 (0.4)	0	0	0	7 (0.3)	0
Congenital	0	0	0	0	0	0	0	0	2 (0.1)	0
Ear/Labyrinth	0	0	0	0	1 (0.2)	0	0	0	2 (0.1)	2 (0.3)
Endocrine	0	0	0	0	0	0	0	0	1 (0.0)	0
Eye	0	0	0	0	1 (0.2)	0	0	0	8 (0.3)	2 (0.3)
Gastrointestinal	1 (7.7)	0	0	0	8 (1.5)	1 (0.5)	1 (7.7)	0	56 (2.4)	20 (2.6)
General/Admin	0	0	5(8.5)	1 (5.3)	22 (4.2)	0	0	0	90 (3.9)	9 (1.2)
Hepatobiliary	0	0	0	0	0	0	0	0	2 (0.1)	2 (0.3)
Immune	0	0	0	0	0	0	0	0	3 (0.1)	3 (0.4
Infections	0	0	5 (8.5)	4 (21.1)	24 (4.5)	12 (6.5)	0	0	200 (8.6)	52 (6.7)
Injury/Poison	0	0	0	1 (5.3)	12 (2.3)	5 (2.7)	1 (7.7)	0	53 (2.3)	18 (2.3)
Investigations	0	0	0	2 (10.5)	3 (0.6)	1 (0.5)	0	0	27 (1.2)	12 (1.6)
Metabolism	0	0	0	0	0	0	0	0	4 (0.2)	2 (0.3)
MSK/CTD	0	0	0	1 (5.3)	2 (0.4)	2 (1.1)	1 (7.7)	0	43 (1.8)	19 (2.5)
Nervous	0	0	2 (3.4)	0	8 (1.5)	2 (1.1)	0	0	52 (2.2)	11 (1.4)
Psychiatric	0	0	0	0	2 (0.4)	0	0	0	15 (0.6)	8 (1.0)
Renal	0	0	0	0	0	1 (0.5)	0	0	7 (0.3)	3 (0.4)
Repro/Breast	0	0	1 (1.7)	0	2 (0.4)	1 (0.5)	0	0	8 (0.3)	4 (0.5)
Resp/Thoracic	1 (7.7)	0	1 (1.7)	0	7 (1.3)	2 (1.1)	0	1 (33.3)	66 (2.8)	13 (1.7)
Skin/SubQ	0	0	2 (3.4)	0	7 (1.3)	2 (1.1)	0	0	41 (1.8)	15 (1.9)
Surgical/Proc	0	0	0	0	3 (0.6)	1 (0.5)	0	0	3 (0.1)	1 (0.1)
Vascular	0	0	1 (1.7)	0	2 (0.4)	0	0	0	8 (0.3)	0

Key: "AI/AN" = American Indian/Alaska Native, "Asian" = Oriental/Asian, "Black" = Black/African American, "H/PI" = Native Hawaiian/ Other Pacific Islander, "White" = White/ Caucasian, "MVA" = received MVA vaccine, "P" = received Placebo

Source: Original BLA 125678/0. Adapted from POX-MVA-013 Clinical Study Report Table 15.3.1.4.2.3, p 1142-1177

6.3.12.3 Deaths

One subject died during MVA-POX-013. The subject, (b) (6) , was a 20-year-old White/Caucasian male with no known past medical or psychiatric history. He was enrolled in Group 1 and received one dose of MVA-BN (Lot 1) on (b) (6) prior to his death by suicide, which occurred (b) (6) days after vaccination on (b) (6) . The family requested that no further contact was made by the trial investigators or staff, so no additional records were collected. The site investigator determined that the patient's death by suicide was unrelated to study vaccine.

Reviewer Comment: *Two additional subjects in this trial attempted suicide without a result of death. The first subject,* (b) (6) *, also randomized to MVA-BN Group 1, was a 28-year-old male with no reported past medical history. The second,* (b) (6) *, was in the Placebo Group (Group 4) and was a 26-year-old female with a known history of anxiety and depression, on multiple anti-depressants. Both events were also determined to be unrelated to study vaccine. See more detailed description of both SAEs in Section 6.3.12.4. Rates of attempted or completed suicide observed in this study, 3/4005 or 0.07/1000 were not above age-adjusted rates of attempted suicide in the United States at the time of the study, which were 0.13/1000 overall or 0.2/1000 for men in 2013-2014 (*https://www.nimh.nih.gov/health/statistics/suicide.shtml*).*

6.3.12.4 Nonfatal Serious Adverse Events

There were 34 non-fatal SAEs reported by 33 subjects during the study (Table 51). The most commonly reported Body System/Organ Class categories for SAEs were Injury, Poisoning and Procedural Complications (n=6), Infections and Infestations (n=6), Psychiatric Disorders (n=4), Nervous System Disorders (n=5), Gastrointestinal Disorders (n=5) and Pregnancy, Puerperium and Perinatal Conditions (n=4). SAEs which were reported in more than one subject include appendicitis (4, one prior to vaccination during screening period), spontaneous abortion/fetal death (4), attempted suicide (2), depression (2) and concussion (2). SAEs were spread across all study groups, with the highest number occurring in Group 1 (10), followed by Group 4/Placebo (9), Group 3 (8) and Group 2 (7). Nineteen SAEs occurred during the active treatment phase of the study (Visit 1-Visit 5), one during screening and the remaining fourteen during follow-up. SAEs occurred slightly more frequently in women (19) versus men (14). All but four of the SAEs (concussion, GERD, pelvic fracture and new onset neurological symptoms) occurred in White/Caucasian subjects.

Only two SAEs, Seizure ((b) (6) , Placebo) and Spontaneous Abortion ((b) (6) , Group 3) were reported as "Possibly Related" to study vaccine. Three SAEs were recorded as "Unlikely Related": Thrombophlebitis ((b) (6) , Group1), Hiatal Hernia ((b) (6) , Group 1) and Spontaneous Abortion ((b) (6) , Placebo). The remainder of the SAEs were determined by the sponsor to be unrelated to vaccination.

There were 24 women total who became pregnant during the study and follow-up period, 14 of whom received MVA-BN. Five women gave birth to healthy infants. Three women had elective (non-medically indicated) abortions. Four women (17% of pregnancies during study, 0.19% all women in study) reported negative fetal outcomes: In the MVA-BN groups, one had fetal death (~12 weeks EGA) at (b) (6) days after last vaccine dose, EDC > 28 days after 2nd dose and one had a SAB at (b) (6) days after last MVA-BN dose, EDC (b) (6) days after 2nd dose. In the Placebo group, two had SABs: one at (b) (6) days after 2nd dose with EDC (b) (6) days after 2nd vaccination and the other at (b) (6) days after the 2nd vaccination. Four women were lost to follow-up and the outcomes of their pregnancy are unknown.

Table 51: Subjects with Serious Adverse Events in POX-MVA-013

Subject (Group)	SAE	Days Since Last Vaccine	Relation-ship	Outcome	Comments
(b) (6)	Thrombophlebitis	152	Unlikely	Resolved	27yoM with h/o DVT with superficial saphenous vein thrombus
	Headache	28 (Study Day 58)	Unrelated	Resolved	32yoF with h/o migraines, new headache, normal MRI.
	Perforated appendicitis	177	Unrelated	Resolved	41yoF with perforated appendicitis remote from vaccination.
	Fetal death	(b) (6)	Unrelated	Resolved	22yoFh/o 2 previous incomplete pregnancies, with EDC >28 days from last vaccine with SAB at ~12 weeks.? IVS thrombosis vs hemorrhage.
	Suicide attempt	36	Unrelated	Resolved	28yoM with no recorded medical hx, attempted suicide by OD with heroin and benzos following fight with fiancée. Recovered after Narcan. Did not receive 2nd dose.
	Chest pain	22 (Study Day 50)	Unrelated	Resolved	31yoF with DM, HTN, thyroid disease, bipolar depression with sharp L sided chest pain and associated SOB 22 days after 2nd dose. Spontaneously resolved. Neg ECG.
	Concussion	131	Unrelated	Resolved	25yoM with no PMH with concussion and facial injury 2/2 to rugby.
	Hodgkin's Lymphoma	121	Unrelated	Resolved	26yoF with neck swelling, chest, pain,

Subject (Group)	SAE	Days Since Last Vaccine	Relation-ship	Outcome	Comments
					fever, night sweats, dx with Hodgkin's Lymphoma
(b) (6)	GERD	14	Unrelated	Resolved	39yoM p/w chest pain and rash; normal ECG and troponin (0.01, <0.05 ref); normal echo; also dx with hip pain, HTN and MRSA skin infection
	Hiatal hernia	23	Unlikely	Resolved	26yoF with IBS, PCOS, asthma, depression, h/o cervical cancer, dx with hiatal hernia 23 days after 1st dose.
	Appendicitis	-25	N/A	Resolved	Occurred after screening and subject did not receive vaccine.
	Ataxia	0 (Study Day 47)	Unrelated	Resolved	35yoM no PMH with moderate ataxia, weakness, nausea/emesis on day of 2nd vaccination. Neg labs, MRI brain. Unclear cause but resolved.
	Pancreatitis	41 (76)	Unrelated	Resolved	35yoM h/o HTN, EtOH-induced pancreatitis, PTSD with pancreatitis (clinical/radiographic dx) two days after binge drinking episode.
	Depression	8	Unrelated	Resolved	26yoF h/o allergies, resolved WPW, admitted for observation due to depression 8 days after 1st vaccine. No further doses given.
	Thermal burn	20	Unrelated	Resolved	38yoM with no PMH with partial thickness burn to R arm, sustained while siphoning gasoline from truck 20d after 2nd dose. + tox screen for meth.
	Concussion	8	Unrelated	Resolved	22yoM with no PMH with concussion and scalp laceration 2/2 to high speed MVA 8 days after 1st vaccine dose.

Subject (Group)	SAE	Days Since Last Vaccine	Relation-ship	Outcome	Comments
(b) (6)	Appendicitis	20	Unrelated	Resolved	19yoM with appendicitis 20 days after 1st vaccine.
	Colitis	16	Unrelated	Resolved	31yoM with recent tooth abscess, dx with rectal bleeding and colitis 17 days after 1st vaccine. No infectious cause identified. Resolved after 4 days with abx.
	Appendicitis	19 (Study Day 49)	Unrelated	Resolved	33yoF dx with appendicitis 19 days after 2nd vaccine.
	Syncope	23 (Study Day 59)	Unrelated	Resolved	31yoF with no PMH admitted for syncope of unknown cause 23 days after 2nd vaccine.
	Limb abscess	21	Unrelated	Resolved	31yoF with h/o mood disorder/anxiety with deep L hand MSSA abscess dx 21d after 1st vaccine.
	Gastric ulcer Nephrolithiasis	30 30	Unrelated Unrelated	Resolved Resolved	40yoM with no MH admitted with gastric ulcer and kidney stone 30 days after 2nd dose. No hospital records available.
	Abortion spontaneous	(b) (6)	~~Unlikely~~ Possibly	Resolved	23yoF with spontaneous incomplete abortion (b) (6) days after 2nd dose. EDC (b) (6) days after 2nd vaccine.
	Meningitis Meningitis	78 (Study Day107) 109 (Study Day138)	Unrelated Unrelated	Resolved Resolved	23yoF with no MH who had 2 meningitis episodes (or 1 episode and a "relapse") with no additional details available, 78 days after 2nd dose and 109 days after 2nd dose.
	Excoriation	26	Unrelated	Resolved	26yoM with multiple abrasions following motorcycle accident
	Depression Suicide attempt	99 99	Unrelated Unrelated	Resolved Resolved	26yoF with PMH depression/anxiety with worsening depression x4 mo and suicide attempt (Soma OD) after argument with husband. Did not receive 2nd dose.
	Ankle fracture	57 (Study Day 86)	Unrelated	Resolved	29yoM with no MH with R ankle fracture sustained during martial arts

Subject (Group)	SAE	Days Since Last Vaccine	Relation-ship	Outcome	Comments
(b) (6)	Pelvic fracture	2	Unrelated	Recovered with sequelae	28yoF with h/o depression, insomnia, with pelvic fracture 2/2 to being hit by car 2 days after 1st dose. No 2nd dose given.
	Spontaneous abortion	(b) (6) (Study Day (b) (6))	Unrelated	Resolved	23yoF with no PMH with EDC (b) (6) days after 2nd dose of vaccine and SAB (b) (6) days after 2nd dose.
	Spontaneous abortion	(b) (6) (Study Day (b) (6))	Unlikely	Resolved	29yoF with no MH with SAB (b) (6) days after 2nd dose of placebo.
	Neurological symptom	92	Unrelated	Resolving	34yoF with h/o IHI with new blurry vision, emesis, AMS and hemiparalysis 63 days after 2nd dose. Found to have abnormal EEG of L hemisphere.
	Convulsion	5	Possible	Resolved	24yoM with undisclosed history of childhood seizures (last at 19) with new generalized seizure.

Source: Original BLA 125678/0. Adapted from POX-MVA-013 Clinical Study Report Table 24 (p 92) and Section 15.3.6 "Narratives of Deaths, other Serious and Certain Other Significant Adverse Events" (p1536-1603)

***Reviewer Comment**: IR (#10 sent to sponsor on 14 January 2019) re: 2 more events, for subjects* (b) (6) *(TMJ pain and Worsening Cholelithiasis) which resulted in hospitalization but were not flagged as SAEs in the database. Per sponsor response ("Response to FDA Request for Information #10", received 28 January 2019), both were pre-existing conditions and were treated with a planned surgery and an outpatient surgery, not true hospital admissions and therefore were not considered serious.*

***Reviewer Comment:** There were 4 episodes of spontaneous abortion/fetal loss during the study and they were evenly distributed between vaccine (1 in Group 1 and 1 in Group 3) and placebo groups (2 in Placebo). Appendicitis and depression/suicide attempt were also reported by multiple subjects. All four cases of appendicitis were reported in vaccine treatment groups, however only 3 cases occurred after a study vaccine had been received, leading to an incidence of 0.1% or 1 in 1000 subjects. This is the same as the background rate of appendicitis in the United States, which is 1-2 cases per 1000 persons[14, 15]. The psychiatric SAEs were evenly distributed across treatment groups. There was 1 completed suicide (not included in Non-Fatal SAE counts above), 1 suicide attempt and 1 hospitalization for depression in Groups 1-3 (n=3, 0.1%) and 1 hospitalization for depression and 1 suicide attempt in the Placebo group (n=2, 0.2%). These findings are most reflective of a population of young, otherwise*

physically healthy subjects and not indicative of any significant safety signals for the MVA-BN vaccine in this study.

Reviewer Comment: *In this reviewer's assessment of the unblinded study data, the two reported possibly related SAEs ((b) (6) with a spontaneous abortion (b) (6) days following a dose of MVA-BN, which was received (b) (6) days after the estimated date of conception and (b) (6) with a convulsion 5 days after placebo) are unrelated to vaccination based on timing (SAB) and lack of a plausible physiologic relationship between convulsion and a tris-saline placebo injection (convulsion). I agree with the other assessments of causality as listed above.*

6.3.12.5 Adverse Events of Special Interest

A total of 8 of 3003 subjects (0.26%) in the MVA-BN groups (1-3) and 1 of 1002 subjects (0.1%) in the Placebo group (4) reported AESIs (Table 52). Events occurred in 2 subjects in Group 1, 5 subjects in Group 2 and 1 subject in Group 3. All but one of these events (tachycardia in a subject in Group 2) occurred after the first vaccination. Overall, 5 subjects who experienced AESIs were referred to a cardiologist for additional evaluation. Both subjects who were reported as having elevated troponin I levels had repeat testing as follow-up.

Table 52: Treatment Emergent Adverse Events of Special Interest by Preferred Term (Full Analysis Set) in POX-MVA-013

Subject Number	Treatment Group	AESI (PT)	Relative Day of Onset	Relationship	Outcome
(b) (6)	1	Troponin I increased	14	Unlikely	Recovered/Resolved
	1	Wolff-Parkinson-White syndrome	14	Unrelated	Recovered/Resolved
	2	Troponin I increased	15	Unlikely	Recovered/Resolved
	2	Supraventricular extrasystoles	28	Unrelated	Recovered/Resolved
	2	Right Bundle Branch Block	15	Possible	Not recovered or resolved
	2	Tachycardia	17	Unrelated	Recovered/Resolved
	2	ECG ST segment abnormal	12	Unrelated	Unknown/Lost to follow-up
	3	Possible acute pericarditis	23	Possible	Recovered/Resolved
	4	Right Bundle Branch Block	16	Unrelated	Recovered/Resolved

Source: Original BLA 125678/0. Adapted from POX-MVA-013 Clinical Study Report, p. 96.

Reviewer Comment: *Overall, this reviewer agrees with the applicant's assessment of causality for the AESIs listed above. Please see section 8.4.8 for further discussion of the case of possible pericarditis reported for subject (b) (6) .*

Cardiac Symptoms and ECGs: Routine ECGs were performed on all subjects at baseline and all but two enrolled subjects (N=3928) at Visit 2 (two weeks after the first vaccination). Seven percent of subjects had a clinically significant abnormal ECG at baseline in the combined vaccine groups (N=216) compared to 6.2% in the placebo group (Table 53). The percentage of subjects with a clinically abnormal ECG went up slightly at Visit 2 for all groups (7.9% in Groups 1-3, 6.8% Group 4). However, most subjects with abnormal ECGs at Visit 2 also had abnormal ECGs at screening/baseline and therefore their Visit 2 findings were not considered a change. A total of 89 (3.0%) subjects who received MVA-BN (37 [3.7%] from Group 1, 28 [2.9%] from Group 2 and 24 [2.4%] from Group 3) had a change from normal/normal variant to abnormal ECG at Visit 2. A similar percentage (2.6%, N=26) of subjects from the placebo group also developed new abnormal ECG changes at Visit 2. In the limited number of MVA-BN subjects who had an ECG performed at Visit 4, 4.4% (range 2.0-7.3%, n=6) of subjects developed a new abnormal ECG finding compared to baseline. A higher percentage of subjects in the placebo group (9.3%, n=4) demonstrated a new abnormal finding on ECG at Visit 4 compared to baseline. All abnormal ECG findings were evaluated further by the investigator and only 3 subjects at Visit 2 (two in Group 2 and one in Group 4/Placebo) were adjudicated as having clinically significant ECG abnormalities.

During the period following the first vaccination, 5 symptomatic or electrocardiographic AESIs were reported in the MVA-BN groups and 1 AESI was reported for Placebo. One subject in Group 1 ((b) (6)) was found to have Wolff-Parkinson-White syndrome on ECG. One subject each in Group 2 were found to have right bundle branch block ((b) (6)), supraventricular extra-systolic activity ((b) (6)) and ST segment abnormalities on ECG ((b) (6)). One subject in Group 3 presented with possible acute pericarditis ((b) (6)) and one subject in Group 4 was diagnosed with right bundle branch block ((b) (6)). The episode of right bundle branch block in subject (b) (6) and the possible acute pericarditis in subject (b) (6) were both considered possibly related to MVA-BN.

During the period following the second vaccination, only 1 AESI, tachycardia, was reported in subject (b) (6) in Group 2. Routine ECGs were not performed during the second vaccination period.

Table 53: Electrocardiogram (ECG) Screening and Results by Treatment Group and Visit in POX-MVA-013

Category	Group 1 (N=999)	Group 2 (N=1005)	Group 3 (N= 999)	Combined Group 1-3	Group 4 (N=1002)
Screening ECG Performed (Yes)	999 (100%)	1005 (100%)	999 (100%)	3003 (100%)	1002 (100%)
Screening ECG Normal/Normal Variant (NV)	917 (91.8%)	927 (92.2%)	939 (94.0%)	2783 (92.7%)	939 (93.7%)
Screening ECG Abnormal	80 (8.0%)	77 (7.7%)	59 (5.9%)	216 (7.2%)	62 (6.2%)

Category	Group 1 (N=999)	Group 2 (N=1005)	Group 3 (N= 999)	Combined Group 1-3	Group 4 (N=1002)
Visit 2 ECG Performed (Yes)	986 (98.7%)	977 (97.2%)	984 (98.5%)	2947 (98.1%)	981 (97.9%)
Visit 2 ECG Normal/NV	892 (90.5%)	890 (91.1%)	914 (92.9%)	2696 (91.5%)	909 (92.7%)
Visit 2 ECG Abnormal	91 (9.1%)	82 (8.2%)	65 (6.5%)	238 (7.9%)	68 (6.8%)
Normal Baseline toAbnormal Visit 2	37 (3.7%)	28 (2.9%)	24 (2.4%)	89 (3.0%)	26 (2.6%)
Visit 4 ECG Performed (Yes)	41 (4.1%)	51 (5.1%)	44 (4.4%)	136 (4.5%)	43 (4.3%)
Visit 4 ECG Normal/NV	33 (80.5%)	44 (86.3%)	41 (93.2%)	118 (86.8%)	36 (83.7%)
Visit 4 ECG Abnormal	8 (19.5%)	7 (13.7%)	3 (6.8%)	18 (13.2%)	6 (13.9%)
Normal Baseline to Abnormal Visit 4	3 (7.3%)	1 (2.0%)	2 (4.5%)	6 (4.4%)	4 (9.3%)

Source: Original BLA 125678/0. POX-MVA-013 Clinical Study Report, Adapted from Tables 15.3.4.2 and 15.3.4.3 on p. 1525-1529

Reviewer Comment: *Additional adverse events reported from the Cardiac Disorders SOC which were not included in the sponsor's summary of AESIs include: RBBB in subject* (b) (6) *(Group 3), which occurred on day 25 (screening ECG), tachycardia in subject* (b) (6) *(Group 3) which occurred on study day 29, sinus tachycardia in subject* (b) (6) *(Group 3) which occurred on study day 15-17, palpitations in subject* (b) (6) *(Group 2) which occurred on study day 45-47 and AV block first degree in subject* (b) (6) *(Group 1) on study day 13. These were determined by the DSMB to not be clinically significant or represent true "cardiac signs or symptoms".*

Troponin I: All subjects from the FAS (N=4005) had troponin I collected at baseline. Most subjects had troponin I collected at Visit 2, 2 weeks after the first vaccine dose, however 2.8% (N=114 total) of subjects did not have data for troponin I from this visit. Most subjects (99.6%) had troponin I levels below the lower limit of the normal range (0.03) at baseline. Ten subjects had values within the normal range (0.03-0.05) and 3 subjects had values above 0.05 ((b) (6) from Group 1 and (b) (6) [both Troponin I (b) (4) and (b) (4) HBT]).

At Visit 2, there were 3898 troponin I values reported. Most (N= 3885, 99.6%) were below or within the reference range (N=8, <0.1%). Three (<0.1%) subjects ((b) (6)) had 5 levels above the reference range. All of these values represented a rise over baseline troponin I levels. All subjects with elevated levels were in MVA-BN treatment groups (two in Group 1, one in Group 2).

Reviewer Comment: *As previously mentioned, subject* (b) (6) *had an elevated troponin I at baseline (0.08) which rose at Visit 2 (0.10) and continued to rise (0.17) at an unscheduled follow up visit held 9 days later. This subject's troponin I normalized (<0.01) at an unscheduled follow-up visit 2 months later. Subject*

(b) (6) *also had elevated troponin I (0.09) at baseline (coded as "unscheduled visit" for screening visit), which then increased to 0.13 at Visit 2. Troponin I fell within the reference range (0.02) at a follow-up visit 5 days after initial visit and he did receive a 2nd dose of vaccine. Subject* (b) (6) *had a troponin I within reference range at baseline and had an elevated troponin I at Visit 2. No follow-up troponin I value is provided.*

Some subjects had additional troponin I levels collected at Visits 3 (N=2), 4 (N=121) and 5 (N=9). All values collected at Visits 3-5 were either below or within the reference range.

Subjects also had troponin I collected at some visits outside of the standard study schedule for medical reasons ("unscheduled visits"). There were 38 troponin I (and 1 troponin I HBT) levels collected at unscheduled visits during the screening and study period. The highest number of troponin I tests collected during unscheduled visits occurred in the Placebo group (N=13), followed by Groups 2, 3 and 1 (N= 10, 9 and 7). The only values that were elevated (N=2) at unscheduled visits were for subject (b) (6) from Group 2, who had an elevated troponin I at baseline.

Reviewer Comment: *Some screening/baseline visits were coded as "unscheduled" for unclear reasons.*

No significant difference in troponin I values was noted amongst treatment groups, including placebo, during the study period (P>0.06). None of the elevated troponin I values were considered by the applicant to be related to the MVA-BN vaccine.

Reviewer Comment: *This reviewer agrees with the applicant's assessment regarding causality of troponin I elevations in subjects* (b) (6) *, given that both subjects had elevated troponin I at baseline and, specifically for subject* (b) (6) *, an alternative explanation (marathon training) for elevated troponin I was elicited. Subject* (b) (6) *did not have an abnormal troponin I at baseline and did not have any further documentation or explanation regarding his elevated level at visit 2. This subject did not have follow-up lab work or evaluation and as such, it is difficult to rule out that this troponin elevation was at least possible related to MVA-BN. None of these subjects reported clinical symptoms that would be concerning for myopericarditis.*

6.3.12.6 Clinical Test Results

There were 69 abnormal laboratory values reported by subjects in the study (Table 54). The most common abnormal laboratory test was urine beta HCG, which occurred in 18 subjects. The next most common was elevated AST in 17 subjects, followed by hyperkalemia in 12 subjects and elevated ALT in 11 subjects. Abnormal values were fairly similar across MVA-BN treatment groups

and the occurrence of positive beta-HCG, elevated AST and ALT and elevated potassium was higher in the placebo group compared to the MVA-BN groups.

Table 54: Clinical Lab Abnormalities by Treatment Group and Preferred Term in POX-MVA-013

Lab Abnormalities	Group 1 (N=999), n (%)	Group 2 (N=1005), n (%)	Group 3 (N=999), n (%)	Groups 1-3 (N=3003), n (%)	Group 4 (N=1002), n (%)
Positive Beta-HCG	3 (0.3%)	4 (0.4%)	3 (0.3%)	10 (0.3%)	8 (0.7%)
Elevated AST	2 (0.2%)	3 (0.3%)	2 (0.2%)	7 (0.2%)	10 (0.9%)
Elevated potassium	2 (0.2%)	2 (0.2%)	3 (0.3%)	7 (0.2%)	5 (0.5%)
Elevated ALT	2 (0.2%)	0	1 (0.1%)	3 (0.1%)	8 (0.7%)
Decreased neutrophils	2 (0.2%)	3 (0.3%)	0	5 (0.1%)	0
Elevated Troponin I	2 (0.2%)	1 (0.1%)	0	3 (0.1%)	0
Decreased lymphocytes	1 (0.1%)	0	1 (0.1%)	2 (<0.1%)	0
Elevated sodium	0	0	1 (0.1%)	1 (<0.1%)	0

Source: Original BLA 125678/0. Adapted from POX-MVA-013 Clinical Study Report POX-MVA-013, Tables 15.3.3.5 and 15.3.3.6, p 1392-1420
N=total number of subjects in the specified group, n=number of subjects with specified events

Abnormal laboratory values and investigations listed as treatment emergent AEs include 38 AEs from 30 subjects in the MVA-BN groups and 20 AEs from 15 subjects in the placebo group (Table 55). The most commonly reported abnormalities were elevated AST (n=10, 0.3%), elevated ALT (n=8, 0.3%) and increased blood pressure (n=4, 0.1%) in the MVA-BN group and in the placebo group (elevated AST in 5 subjects, elevated ALT in 5 subjects and elevated blood pressure in 2 subjects). Only one abnormal lab value, an elevated AST in a subject in Group 3, was Grade 3.

Table 55: Treatment Emergent Adverse Events of SOC "Investigations" by Treatment Group and Preferred Term in POX-MVA-013

SOC: Investigations Preferred Terms	Group 1 (N =999) Subjects (%) /Events	Group 2 (N=1005) Subjects (%) /Events	Group 3 (N=999) Subjects (%) /Events	Combined Groups 1-3 (N=3003) Subjects (%) /Events	Group 4 (N=1002) Subjects (%) /Events
ALT increased	3 (0.3)/ 3	2 (0.2)/ 2	3 (0.3)/ 4	8 (0.3)/ 9	5 (0.5)/ 5
AST increased	3 (0.3) /3	1 (0.1)/ 1	6 (0.6)/ 6	10 (0.3)/ 10	5 (0.5)/ 5
Blood alkaline phosphatase increased	1 (0.1)/ 1	0	0	1 (0.0)/ 1	0
Blood creatinine increased	0	0	1 (0.1)/ 1	1 (0.0)/ 1	1 (0.1)/ 1
Blood pressure increased	1(0.1)/ 1	1 (0.1)/ 1	2 (0.2)/ 2	4 (0.1)/ 4	2 (0.2)/ 3

SOC: Investigations Preferred Terms	Group 1 (N =999) Subjects (%) /Events	Group 2 (N=1005) Subjects (%) /Events	Group 3 (N=999) Subjects (%) /Events	Combined Groups 1-3 (N=3003) Subjects (%) /Events	Group 4 (N=1002) Subjects (%) /Events
Blood testosterone decreased	0	0	0	0	1 (0.1)/ 1
ECG ST segment abnormality	0	1 (0.1)/ 1	0	1 (0.0)/ 1	0
ECG T wave abnormality	1 (0.1)/ 1	0	0	1 (0.0)/ 1	0
Hemoglobin decreased	0	1 (0.1)/ 1	0	1 (0.0)/ 1	0
Hepatic enzyme increased	0	0	0	0	2 (0.2)/ 2
Liver function test abnormal	0	1 (0.1)/ 1	1 (0.1)/ 1	2 (0.0)/ 2	2 (0.2)/ 2
Lymph node palpable	0	1 (0.1)/ 1	0	1 (0.0)/ 1	0
Lymphocyte count increased	0	0	1(0.1)/ 1	1 (0.0)/ 1	0
QRS axis abnormal	0	0	1 (0.1)/ 1	1 (0.0)/ 1	0
Transaminases increased	0	0	1 (0.1)/ 1	1 (0.0)/ 1	1 (0.1) /1
Troponin I increased	1 (0.1)/ 1	1 (0.1)/ 1	0	2 (0.0)/ 2	0
Tuberculin test positive	1 (0.1)/ 1	0	0	1 (0.0)/ 1	0
Weight increased	1(0.1)/ 1	0	0	1 (0.0)/ 1	0
Total	9 (0.9)/ 12	8 (0.8)/ 9	13 (1.3)/ 17	30 (1.0)/ 38	15 (1.5)/ 20

Source: Original BLA 125678/0. Adapted from POX-MVA-013 Clinical Study Report, Table 15.3.1.4.2, p 1074

6.3.12.7 Dropouts and/or Discontinuations

Overall, twenty subjects (0.5%) were discontinued from the study or from further vaccination due to an adverse event. All but one of these subjects (19/20) experienced an adverse event within 28 days of the first vaccination, resulting in discontinuation from the 2nd dose. Discontinuations were highest in Group 2 (n=8), but otherwise evenly distributed between treatment groups (Group 1: 4 discontinued subjects due to AE, Group 3: 4 discontinued subjects due to AE, Group 4: 3 discontinued subjects due to AE).

- Group 1:
  - Subject (b) (6) - discontinued after 2nd dose due to adverse event (positive PPD; unrelated to study vaccine)
  - Subject (b) (6) - discontinued after 1st dose due to adverse event (mild arthralgia at D20; unlikely related)
  - Subject (b) (6) - discontinued after adverse event after 1st dose (suicide attempt; unrelated)
  - Subject (b) (6) - after 1st dose (death by suicide; unrelated)
  - Subject (b) (6) - after 1st dose (WPW on ECG, also considered AESI; unrelated)
- Group 2:
  - Subject (b) (6) - after 1st dose (elevated AST and ALT on D17; unlikely related)
  - Subject (b) (6) - after 1st dose (MRSA SSTI on D26, unrelated)

    - Subject (b) (6) -after 1st dose (persistent injection site induration, hyperpigmentation D1-30; likely related)
    - Subject (b) (6) -after 1st dose (RBBB on D16; possibly related)
    - Subject (b) (6) - after 1st dose (non-specific ST elevation on D13unlikely related)
    - Subject (b) (6) - after 1st dose (pharyngitis on D21; unrelated)
    - Subject (b) (6) - after 1st dose (sinusitis on D12; unrelated)
    - Subject (b) (6) - after 1st dose (kidney stone and UTI on D22; unrelated)
- Group 3:
    - Subject (b) (6) - after 1st dose (cardiac symptoms,
    - Subject (b) (6) - after 1st dose (moderate urticaria on D3; related)
    - Subject (b) (6) – after 1st dose (elevated AST and ALT on D14; unlikely related)
    - Subject (b) (6) - after 1st dose (mild pruritus first presenting on D8; unlikely related)
- Group 4 (Placebo)
    - Subject (b) (6) - after 1st dose (pelvic fracture on D3; unrelated)
    - Subject (b) (6) - after 1st dose (axillary cellulitis on D28);
    - Subject (b) (6) - after 1st dose (seizures on D6)

6.3.13 Study Summary and Conclusions

This study was a randomized, double-blind, placebo-controlled Phase 3 trial to assess the consistency of safety and immunogenicity of two doses of MVA-BN from three consecutively produced lots in healthy, young, low-cardiac risk, vaccinia-naïve subjects. The study design was acceptable for a lot consistency study. The quality of the data generally appears adequate.

The equivalence of immunogenicity parameters amongst the three consecutive vaccine lots was assessed in this study. The ratio of vaccinia specific $PRNT_{50}$ titers between all lots was within the pre-specified equivalence range (0.5-2.0), demonstrating consistency between lots.

Most MVA-BN recipients reported at least one adverse event, most of which were mild or moderate in severity and were primarily injection site reactions. Incidence and type of TEAEs and SAEs were similar between subjects who received the vaccine and placebo. Only one SAE (spontaneous abortion) in a subject who received MVA-BN was reported as at least possibly related to the study vaccine; however, it is unlikely to this reviewer that this event was related to study vaccination as previously discussed. There was a low incidence of AESIs in both MVA-BN and placebo groups overall, though slightly higher in the group receiving MVA-BN (0.26% vs 0.1%). Of note, ECGs and troponin I levels were only obtained after the first dose so additional subclinical AESIs may have been missed. One subject died (from suicide) in this study, which was not considered related to the study vaccine.

Overall, this study demonstrates a consistent and adequate safety and immunogenicity profile in three consecutively produced lots of MVA-BN in healthy, low cardiac risk, vaccinia naïve adults. No significant increase in risk of new onset cardiac symptoms, lab or electrographic abnormalities was noted in this study. A post-marketing study for potential cardiac adverse events is planned, however only in the setting of a smallpox outbreak event, and as such future data on the risk of possible cardiac symptoms in the setting of MVA-BN will be limited. A detailed discussion of the overall risk/benefit of giving two doses of MVA-BN to individuals at high risk for smallpox exposure is discussed in Section 11.

7. Integrated Overview of Efficacy

Only one effectiveness study was conducted in the drug development program; therefore, an ISE was not necessary.

8. Integrated Overview of Safety

8.1 Safety Assessment Methods

In all studies, safety assessments were performed throughout the studies. Solicited systemic and injection-site adverse reactions were collected via diary card following each vaccination for 7 days for all studies except for studies POX-MVA-002, -006, -009, -028, -029 and -030 in which solicited adverse reactions were collected for 14 days after each vaccination. Unsolicited non-serious adverse events were collected for 28 days after each vaccination, and SAEs and AESI for at least 6 months up to 24 months after the last vaccination. AESIs included monitoring ECG (except for POX-MVA-004 and -03X) and troponin at 7 to 15 days after vaccination and cardiac related symptoms and signs throughout studies. In some studies, cardiac related events were not categorized as AESIs but were designated as cardiac disorder or events (POX-MVA-028, -029, -030, -036, and -03X) or other significant adverse events (POX-MVA-009). In this review, all cardiac related events including abnormal ECG and troponin are categorized as AESIs.

BN submitted two integrated summaries of safety (ISS): a pooled safety analysis of 12 clinical studies in which all subjects were exposed to the LF formulation administered in the regimen proposed for licensure (referred to as the Main ISS in this review) and a broader pooled safety analysis (referred to as the ISS in this review) of all 22 clinical studies regardless of dose level, route of administration and formulation in order to increase the likelihood of detecting potentially rare SAEs and AESIs.

The Main ISS will provide in-depth analyses of solicited AEs and unsolicited non-serious AEs among the subjects who received the to-be-licensed regimen of

MVA-BN, while the ISS will focus on SAEs and AESIs reported from all the 22 clinical trials under the product development program.

For studies in which study subjects received both MVA-BN and ACAM2000 or Dryvax sequentially, events occurred after the subjects received ACAM2000 or Dryvax following MVA-BN vaccination were excluded from the pooled analyses of MVA-BN.

8.2 Safety Database

8.2.1 Studies/Clinical Trials Used to Evaluate Safety

Clinical trials included in the Main ISS are summarized and presented in Table 56. All subjects in the Main ISS pooled population received at least one dose of the LF formulation of MVA-BN. Among the 5110 vaccinia-naïve subjects including healthy, AD and HIV infected subjects who received at least one dose of MVA-BN, 4861 subjects (95.1%) received both doses. All the 409 vaccinia-experienced received one dose of MVA-BN.

Table 56: Summary of Studies Included in the Main Pooled Safety Data Analysis (Main ISS)

Study[a]	**Vaccinia-Naïve Healthy Subjects**	**Vaccinia-Naïve Healthy Subjects**	**Vaccinia-Naïve Healthy Subjects**	**Vaccinia-Naïve AD Subjects**	**Vaccinia-Naïve HIV Infected Subjects**	**Vaccinia-Experienced Healthy Subjects**
	MVA-BN	ACAM2000	Placebo	MVA-BN	MVA-BN	MVA-BN
005	183	0	181	0	0	200
006	220	213	0	0	0	0
008	282	0	0	350	0	0
009	66	0	0	0	0	0
011	88	0	0	0	352	0
013	3003	0	1002	0	0	0
023	0	0	0	0	0	152
024	0	0	0	0	0	57
027	327	0	0	0	0	0
028	45	0	0	0	0	0
029	167	0	0	0	0	0
037	0	0	0	0	27	0
Total Subjects	**4381**	**213**	**1183**	**350**	**379**	**409**

Source: Adapted from Table 1 of Main ISS (page 11), Module 5.3.5.3 under STN125678/0.7.
[a]All studies were designated as POX-MVA- followed by three digits, only the three digits for each study are displayed.
AD=atopic dermatitis

Subjects who received at least one dose of MVA-BN regardless of doses, regimens and formulations from the all 22 studies were included in the broader pooled ISS. Numbers of subjects stratified by vaccine formulations and subject health status are presented in Table 57.

Table 57: Summary of Studies Included in the Pooled ISS

Study	Healthy Subjects	HIV Infected Subjects	Subjects with AD	Others[a]	Total
(b) (4) **Formulation**					
POX-MVA-002	75	0	0	0	75
POX-MVA-004	164	0	0	0	164
POX-MVA-007	15	0	31	14	60
POX-MVA-010	60	91	0	0	151
POX-MVA-027	324	0	0	0	324
POX-MVA-029	165	0	0	0	165
POX-MVA-036	435	0	0	0	435
HIV-NEF-004	0	26	0		26
Subtotal (b) (4)	**1238**	**117**	**31**	**14**	**1400**
LF Formulation					
POX-MVA-001	86	0	0	0	86
POX-MVA-005	564	0	0	0	564
POX-MVA-006	220	0	0	0	220
POX-MVA-008	282	0	350	0	632
POX-MVA-009	190	0	0	0	190
POX-MVA-011	97	482	0	0	579
POX-MVA-013	3003	0	0	0	3003
POX-MVA-023	152	0	0	0	152
POX-MVA-024	120	0	0	0	120
POX-MVA-027	327	0	0	0	327
POX-MVA-028	90	0	0	0	90
POX-MVA-029	358	0	0	0	358
POX-MVA-030	0	0	0	20	20
POX-MVA-037	0	87	0	0	87
POX-MVA-03x	22	0	0	0	22
HIV-POL-002	0	10	0	0	10
Subtotal LF	**5511**	**579**	**350**	**20**	**6460**
Total ((b) (4) +LF)	**6749**	**696**	**381**	**34**	**7860**

Source: Adapted from Table 1 of ISS (page 10-11), Module 5.3.5.3 under STN125678/0.7.
[a] Included subjects with allergic rhinitis in POX-MVA-007 and subjects with hematopoietic stem cell transplantation in POX-MVA-030.
AD=atopic dermatitis; (b) (4) ; LF=liquid-frozen

Reviewer's comment: *In total 11 subjects were excluded from the ISS population: 7 subjects from POX-MVA-009 due to receiving both MVA-BN and Dryvax, and 2 subjects ((b) (6)) from POX-MVA-009 and 1 subject each from POX-MVA-028 ((b) (6)) and POX-MVA-029 ((b) (6)) due to incorrect dose of MVA-BN.*

Subjects (b) (6) *were randomized to placebo but received MVA-BN instead. Subject* (b) (6) *was randomized to receive the high dose of MVA-BN but was dosed incorrectly at Day 0 and did not receive the second vaccination. Subject* (b) (6) *also received an incorrect dose at Day 0 and did not receive the second dose. None of these four subjects reported any SAE or*

AESI. Since these four subjects were excluded from their individual study reports and no SAE or AESI was reported from these subjects, it is acceptable not to include them in the ISS.

8.2.2 Overall Exposure, Demographics of Pooled Safety Populations

8.2.2.1 Baseline Demographics of the Pooled Main ISS Population

The baseline demographic subgroup analyses of the pooled Main ISS population from the 12 clinical trials in which the to-be-licensed regimen of MVA-BN was assessed are presented in Table 58.

The overall pooled analyses included 5110 vaccinia-naïve subjects (including 4381 healthy subjects and 729 subjects with AD or HIV infected), and 409 vaccinia-experienced healthy subjects who received at least one dose of MVA-BN LF formulation. Vaccinia-naïve subjects were younger than vaccinia-experienced subjects, and the HIV infected group had higher proportions of older subjects and male subjects compared with other healthy and AD groups.

In the vaccinia-naïve population, subjects in the MVA-BN treated group and those in the placebo group were generally balanced with regards to their basic demographic characteristics (Table 58). In the ACAM2000 treatment group, subjects were dominantly male (86.4%) because ACAM2000 was assessed as a comparator in only one trial in the military population.

There was no placebo control group or ACAM2000 comparator control group in the vaccinia-experienced population.

Table 58: Subgroup Analyses of Baseline Demographic Characteristics of Subjects (Main ISS Population)

Characteristics	**MVA-BN** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Naïve AD Subjects	**MVA-BN** Vaccinia Naïve HIV Subjects	**MVA-BN** Total Vaccinia Naïve Subjects	**Placebo** Vaccinia Naïve Healthy Subjects	**ACAM2000** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Experienced Healthy Subjects
	(N=4381) n (%)	(N=350) n (%)	(N=379) n (%)	(N=5110) n (%)	(N=1183) n (%)	(N=213) n (%)	(N=409) n (%)
Age (Years)							
Mean ± SD	27.3 ± 6.1	27.3 ± 6.3	36.6 ± 8.0	28.0 ± 6.8	27.4 ± 6.2	23.4 ± 4.6	39.3 ± 13.1
18-40	4353 (99.4%)	349 (99.7%)	261 (68.9%)	4963 (97.1%)	1178 (99.6%)	212 (99.5%)	233 (57.0%)
>40	28 (0.6%)	1 (0.3%)	118 (31.1%)	147 (2.9%)	5 (0.4%)	1 (0.5%)	176 (43.0%)
Sex							
Male	2180 (49.8%)	127 (36.3%)	311 (82.1%)	2618 (51.2%)	537 (45.4%)	184 (86.4%)	169 (41.3%)
Female	2201 (50.2%)	223 (63.7%)	68 (17.9%)	2492 (48.8%)	646 (54.6%)	29 (13.6%)	240 (58.7%)
Race							
America Indian or Alaska Native	23 (0.5%)	0 (0.0%)	1 (0.3%)	24 (0.5%)	7 (0.6%)	6 (2.8%)	0 (0.0%)
Asian	113 (2.5%)	51 (14.6%)	2 (0.5%)	166 (3.2%)	20 (1.7%)	12 (5.6%)	3 (0.7%)
Black	715 (16.3%)	33 (9.4%)	135 (35.6%)	883 (17.3%)	184 (15.6%)	40 (18.8%)	2 (0.5%)
Native Hawaiian or Pacific Islander	19 (0.4%)	1 (0.3%)	1 (0.3%)	21 (0.4%)	3 (0.3%)	3 (1.4%)	0 (0.0%)
White	3285 (75.0%)	127 (36.3%)	171 (45.1%)	3583 (70.1%)	951 (80.4%)	136 (63.8%)	404 (98.8%)
Other	223 (5.1%)	138 (39.4%)	69 (18.2%)	430 (8.4%)	18 (1.5%)	16 (7.5%)	0 (0.0%)
Ethnicity							
Hispanic or Latino	642 (14.7%)	135 (38.6%)	71 (18.7%)	848 (16.6%)	109 (9.2%)	40 (18.8%)	0 (0.0%)
Non-Hispanic or Latino	3309 (75.5%)	0 (0.0%)	22 (5.8%)	3331 (65.2%)	893 (75.5%)	173 (81.2%)	57 (13.9%)
Not Reported	430 (9.8%)	215 (61.4%)	286 (75.5%)	931 (18.2%)	181 (15.3%)	0 (0.0%)	352 (86.1%)

Source: Adapted from Tables 1.3.1 and 1.3.2, Appendix 1 to Main ISS, Module 5.3.5.3_Main ISS, STN125678/0.7.
Note: N=Total number of subjects in the specified treatment group; n=number of subjects in the specified subgroup.

8.2.2.2 Baseline Demographics of the Pooled ISS Population

Reviewer's comment: *The baseline demographics for the pooled ISS population was not provided in the original submission. The request for this information was sent to the applicant on March 27, 2019 (IR21). The applicant submitted its response to STN125678/0.29 on April 10, 2019 (Module 1.11.3).*

The baseline demographic characteristics stratified by subgroups of the pooled ISS population from all 22 studies under the drug development program is presented in Table 59.

Demographic characteristics of the subjects treated with MVA-BNA in the broader safety pooled ISS population were similar to those presented in the Main ISS described above.
The overall pooled analyses included 7093 vaccinia-naïve subjects (including 6216 healthy subjects, 381 AD subjects and 478 HIV infected subjects), and 766 vaccinia-experienced subjects (including 532 healthy subjects and 218 HIV infected subjects) who received at least one dose of MVA-BN regardless of formulation. Vaccinia-naïve subjects were younger than vaccinia-experienced subjects, and the HIV infected group had higher proportions of older subjects and male subjects compared with other healthy or AD groups.

Table 59: Subgroup Analyses of Baseline Demographic Characteristics of Subjects (ISS Population)

Characteristics	**MVA-BN** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Naïve AD Subjects	**MVA-BN** Vaccinia Naïve HIV Subjects	**MVA-BN** Total Vaccinia Naïve Subjects	**Placebo** Vaccinia Naïve Healthy Subjects	**ACAM2000** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Experienced Subjects
	(N=6216) n (%)	(N=381) n (%)	(N=478) n (%)	(N=7093) n (%)	(N=1206) n (%)	(N=213) n (%)	(N=766) n (%)
Age (Years)							
Mean ± SD	27.1 ± 6.0	27.1 ± 6.2	36.1 ± 7.8	27.7 ± 6.5	27.4 ± 6.2	23.4 ± 4.6	43.3 ± 12.3
18-40	6163 (99.1)	380 (99.7)	341 (71.3)	6902 (97.3)	1201 (99.6)	212 (99.5)	304 (39.7)
>40	53 (0.9)	1 (0.3)	137 (28.7)	191 (2.7)	5 (0.4)	1 (0.5)	462 (60.3)
Sex							
Male	3069 (49.4)	141 (37.0)	393 (82.2)	3613 (50.9)	552 (45.8)	184 (86.4)	417 (54.4)
Female	3147 (50.6)	240 (63.0)	85 (17.8)	3480 (49.1)	654 (54.2)	29 (13.6)	349 (45.6)
Race							
America Indian or Alaska Native	24 (0.4)	0 (0.0)	1 (0.2)	25 (0.4)	7 (0.6)	6 (2.8)	0 (0.0)
Asian	170 (2.7)	53 (13.9)	2 (0.4)	225 (3.2)	21 (1.7)	12 (5.6)	6 (0.8)
Black	911 (14.7)	34 (8.9)	180 (37.7)	1125 (15.9)	186 (15.4)	40 (18.8)	82 (10.7)
Native Hawaiian or Pacific Islander	23 (0.4)	1 (0.3)	2 (0.4)	26 (0.4)	3 (0.2)	3 (1.4)	0 (0.0)
White	4801 (77.2)	155 (40.7)	222 (46.4)	5196 (73.3)	971 (80.5)	136 (63.8)	649 (84.7)
Other	287 (4.6)	138 (36.2)	71 (14.9)	496 (7.0)	18 (1.5)	16 (7.5)	29 (3.8)
Ethnicity							
Hispanic or Latino	799 (12.9)	135 (35.4)	78 (16.3)	1012 (14.3)	110 (9.1)	40 (18.8)	29 (3.8)
Non-Hispanic or Latino	4529 (72.9)	0 (0.0)	75 (15.7)	4608 (65.0)	915 (75.9)	173 (81.2)	138 (18.0)
Not Reported	888 (14.3)	246 (64.6)	325 (68.0)	1473 (20.0)	181 (15.0)	0 (0.0)	599 (78.2)

Source: Adapted from Tables 5.5.1 and 5.5.2, Appendix 2 to ISS, Module 1.11.3_Response to IR 21, STN125678/0.29.
Note: N=Total number of subjects in the specified treatment group; n=number of subjects in the specified subgroup.

8.2.3 Categorization of Adverse Events

AEs were coded for each study separately using a variety of versions of MedDRA, allocating an SOC and PT for each event. For the pooled analyses, the coding was redone across all studies to standardize them in MedDRA Version 20.0.

8.3 Caveats Introduced by Pooling of Data Across Studies/Clinical Trials

Safety of MVA-BN was assessed at different dose levels, routes of administration, regimens and formulations under the product development program. Pooling of the 12 clinical trials for the Main ISS is supported by the same dose regimen (i.e., to be licensed dose regimen) and similar safety assessment methods and follow-up. The Main ISS will primarily focus on the solicited adverse reactions and unsolicited non-serious adverse events.

To increase the likelihood of detecting potentially rare and serious AEs and AESIs, a broader pooling was also performed to include all subjects of the 22 clinical trials who received at least one dose of MVA-BN regardless dose level, route of administration and formulation. The ISS will focus on the analysis of SAEs and AESIs.

Differences in the safety assessments were identified among these studies:

- Solicited adverse reactions were assessed for 7 days after each vaccination except for 4 studies: POX-MVA-006, -009, -028 and -029. For these 4 studies, solicited adverse events were collected for 14 days after each vaccination.
- Standard 12-lead ECG was performed in all studies except for studies POX-MVA-004 and POX-MVA-03X.
- Six (POX-MVA-001, -03X, -004, -009, -029, and -036) of the 22 clinical trials did not collect any post-vaccination troponin as part of the laboratory, and thus these trials were excluded from troponin analysis.
- Troponin assessment: Troponin was assessed with various methods across the studies, and the majority of studies did not have placebo controls, which made the interpretation of the abnormal troponin difficult. For example, a significant number of subjects including healthy subjects and subjects with AD or HIV infection in studies POX-MVA-008 and POX-MVA-011 showed abnormal troponin after MVA-BN vaccination, while only a few subjects from all other studies showed abnormal troponin following MVA-BN vaccination. The available information could not determine whether higher frequency of abnormal troponin observed in studies POX-MVA-008 and POX-MVA-011 was attributed to the treatment or the troponin assay used in these two studies. Please refer to Section 8.4.8.2 of this review.

8.4 Safety Results

8.4.1 Deaths

Two deaths were reported among 7860 MVA-BN recipients from the 22 clinical trials of the study program. The deaths were not assessed as unrelated to the vaccine. One each was reported from POX-MVA-011 and POX-MVA-013, respectively. Overall, the death rate was low.

Please refer to Section 6.3.12.3 (POX-MVA-013) and Section 9.1.4 (POX-MVA-011) for narratives of these two deaths.

8.4.2 Nonfatal Serious Adverse Events

8.4.2.1 Nonfatal Serious Adverse Event in Main ISS Population

Summary of SAEs in the Main ISS Population

An overview of the pooled analysis of SAEs from the 12 clinical trials is provided in Table 60. The overall proportions of subjects who experienced SAEs were similar between MVA-BN and placebo recipients. The percentages of subjects experiencing SAEs were similar across populations, ranging from 0.9% to 1.2%, with the exception of the HIV-infected population, who experienced SAEs in 4.5% (17 out of 379) subjects. Among all subjects who received at least one dose of MVA-BN, 66 out of 5519 subjects (1.2%) experienced 83 SAEs, compared with 13 out of 1183 subjects (1.1%) who received placebo experienced 16 SAEs.

No HIV-infected, vaccinia experienced subjects were enrolled in the Main ISS population.

Table 60: Percentages of Subjects with Any Serious Adverse Event (SAE) Stratified by Treatment, Health Status and Previous Experience with Smallpox Vaccines (ISS Safety Population)

Treatment/Population	Percentage of Subjects with Any SAE, n (%)
Placebo/Vaccinia Naïve Subjects	
Healthy Subjects (N=1183)	13 (1.1)
MVA-BN/Vaccinia Naïve Subjects	
All Vaccinia Naïve Subjects (N=5110)	61 (1.2)
Healthy Subjects (N=4381)	41 (0.9)
AD Subjects (N=350)	3 (0.9)
HIV Infected Subjects (N=379)	17 (4.5)
ACAM2000/Vaccinia Naïve Subjects	
Healthy Subjects (N=213)	3 (1.4)
MVA-BN/Vaccinia Experienced Subjects	
Healthy Subjects (N=409)	5 (1.2)

Source: Adapted from Tables 4.4.12.1 and 4.4.12.2, Response to IR 10, Module 1.11.3, STN125678/0.14
Note: AD=atopic dermatitis; N=number of subjects in the specified group; n=numbers of subjects with any SAE.

The overall proportion of healthy subjects who experienced SAEs in vaccinia-experienced subjects (1.2%) was numerically higher than that in vaccinia-naïve subjects (0.9%), however, the numbers are too small to make any meaningful comparisons. The proportion of subjects with SAEs was higher among HIV infected individuals (4.5%) compared with healthy subjects. Among HIV infected subjects, 7 subjects experienced SAEs in the SOC of Infections and Infestation, and 5 subjects experienced SAEs in the SOC of Injury, Poisoning and Procedural Complications.

SAEs by SOC and preferred term reported in the Main ISS population are presented in Table 61. No meaningful difference in the percentage of subjects experienced SAE was identified between the placebo group (1.1%) and MVA-BN treatment groups (1.2% each for vaccinia naïve and experienced subjects).

Table 61: Summary of Subjects with Any Serious Adverse Event (≥0.1%) by System Organ Class and Preferred Term (Main ISS Safety Population)

System Organ Class **Preferred Term**	**Placebo** Vaccinia Naïve Healthy Subjects	**ACAM2000** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Naïve Subjects	**MVA-BN** Vaccinia Experienced Healthy Subjects
	(N=1183) **n (%)**	**(N=213)** **n (%)**	**(N=5110)** **n (%)**	**(N=409)** **n (%)**
Any	**13 (1.1)**	**3 (1.4)**	**61 (1.2)**	**5 (1.2)**
Blood and lymphatic system disorders	**0 (0.0)**	**0 (0.0)**	**3 (0.1)**	**0 (0.0)**
Gastrointestinal disorders	**0 (0.0)**	**1 (0.5)**	**7 (0.1)**	**0 (0.0)**
Hemorrhoids thrombosed	0 (0.0)	1(0.5)	0 (0.0)	0 (0.0)
Infections and infestations	**1 (0.1)**	**0 (0.0)**	**15 (0.3)**	**1 (0.2)**
Appendicitis	0 (0.0)	0 (0.0)	4 (0.1)	0 (0.0)
Gastroenteritis	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)
Gastroenteritis salmonella	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Pneumonia	0 (0.0)	0 (0.0)	3 (0.1)	0 (0.0)
Injury, poisoning and procedural complications	**4 (0.3)**	**1 (0.5)**	**11 (0.2)**	**2 (0.5)**
Ankle fracture	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Concussion	0 (0.0)	0 (0.0)	2 (0.0)	1 (0.2)
Lower limb fracture	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Pelvic fracture	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Road traffic accident	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)
Tendon rupture	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)
Skin abrasion	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Musculoskeletal and connective tissue disorders	**0 (0.0)**	**1 (0.5)**	**2 (0.0)**	**0 (0.0)**
Rhabdomyolysis	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)
Neoplasms benign, malignant and unspecified	**2 (0.2)**	**0 (0.0)**	1 (0.0)	**2 (0.5)**
Benign neoplasm of thyroid gland	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Colon cancer	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Prostate cancer	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.5)
Nervous system disorders	**2 (0.2)**	**0 (0.0)**	**6 (0.1)**	**0 (0.0)**
Neurological symptom	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Seizure	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Pregnancy, puerperium and perinatal conditions	**2 (0.2)**	**0 (0.0)**	**3 (0.1)**	**0 (0.0)**
Abortion spontaneous	2 (0.2)	0 (0.0)	2 (0.0)	0 (0.0)
Psychiatric disorders	**2 (0.2)**	**0 (0.0)**	**6 (0.1)**	**0 (0.0)**
Depression	2 (0.2)	0 (0.0)	1 (0.0)	0 (0.0)
Suicide attempt	1 (0.1)	0 (0.0)	1 (0.0)	0 (0.0)
Respiratory, thoracic and mediastinal disorders	**0 (0.0)**	**0 (0.0)**	**3 (0.1)**	**0 (0.0)**
Surgical and medical procedures	**1 (0.1)**	**0 (0.0)**	**2 (0.0)**	**0 (0.0)**
Thyroidectomy	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)

Source: Adapted from Tables 4.4.12.1 and 4.4.12.2, Response to IR 10, Module 1.11.3, STN125678/0.14
Note: N=number of subjects in specific group; n=number of subjects with specific event.

No particular patterns with regard to the nature of the pooled SAEs were observed, and most SAEs occurred in single subjects only. The individual SAEs that occurred in more than 1 subject were appendicitis (in 4 healthy, vaccinia-naïve subjects), pneumonia (in 2 HIV infected, vaccinia naïve subjects, and 1 AD, vaccinia-naïve subject), asthma (one each in a HIV infected, vaccinia-naïve subject, and an AD, vaccinia-naïve subject), meningitis (in 2 healthy, vaccinia-naïve subjects), and spontaneous abortion (in 2 healthy, vaccinia-naïve subjects).

Three SAEs reported by three individuals were considered related to MVA-BN: two healthy, vaccinia-naïve subjects and one HIV-infected, vaccinia-naïve subject. These three SAEs are described in studies POX-MVA-005 (sarcoidosis, Subject ID (b) (6)), POX-MVA-008 (extraocular muscle paresis, Subject ID (b) (6)) and POX-MVA-011 (pneumonia, Subject ID (b) (6))

Reviewer's comment: *This reviewer has reviewed the narratives of the SAEs and concurs with the applicant's causality assessment.*

Subgroup Analyses of SAEs in the Main ISS

Subgroup analyses of SAEs stratified by age, sex, race and ethnicity are presented in Table 62.

For MVA-BN vaccinated vaccinia naïve populations, there was no apparent pattern or meaningful difference in overall SAE frequencies among subpopulations stratified by sex, race and ethnicity. The subgroup of > 40 years-old had numerically higher rate of SAEs. However, all the 5 SAEs reported by this subgroup were from subjects infected with HIV and none of the SAEs were assessed as treatment related.

For MVA-BN vaccinated vaccinia experienced subjects, the overall SAE frequencies varied greatly among the subgroups, except for the ethnicity groups, due to the limited number of SAEs and study sample size. The numerically higher percentages of subjects with SAEs in the subgroups of males and >55 years-old were primarily driven by three cases of prostate cancer in these subgroups. The apparently higher percentage of SAEs in the black subgroup was likely a random effect, and none of these SAEs (one case each of cellulitis, hip arthroplasty, and pneumothorax, and two cases of gastroenteritis) was related to the treatment.

Table 62: Subgroup Analyses of Serious Adverse Events (SAEs) in Pooled Main ISS Population (Safety Population)

Subgroup	Placebo Vaccinia Naïve Healthy Subjects	ACAM2000 Vaccinia Naïve Healthy Subjects	MVA-BN Vaccinia Naïve Subjects	MVA-BN Vaccinia Experienced Subjects
	Placebo (N=1183)	ACAM2000 (N=213)	MVA-BN (N=5110)	MVA-BN (N=409)
Any, n/N (%)	**13/1183 (1.1)**	**3/213 (1.4)**	**61/5110 (1.2)**	**5/409 (1.2)**
By Age (Years)				
18-40, n/m (%)	12/1178 (1.0)	3/212 (1.4)	56/4963 (1.1)	NA
>40, n/m (%)	1/5 (20.0)	0/1 (0.0)	5/147 (3.4)	NA
18-55, n/m (%)	NA	NA	NA	3/352 (0.9)
>55, n/m (%)	NA	NA	NA	2/57 (3.5)
By Sex				
Male, n/m (%)	5/537 (0.9)	3/184 (1.6)	29/2618 (1.1)	3/169 (1.8)
Female, n/m (%)	8/646 (1.2)	0/29 (0.0)	32/2492 (1.3)	2/240 (0.8)
By Race				
American Indian or Alaska Native, n/m (%)	1/7 (14.3)	0/6 (0.0)	0/24 (0.0)	NA
Asian, n/m (%)	0/20 (0.0)	0/12 (0.0)	1/166 (0.6)	0/3 (0.0)
Black, n/m (%)	1/184 (0.5)	1/40 (2.5)	8/883 (0.9)	0/2 (0.0)
Native Hawaiian or Other Pacific Islander, n/m (%)	0/3 (0.0)	0/3 (0.0)	0/21 (0.0)	NA
White, n/m (%)	11/951 (1.2)	2/136 (1.5)	46/3583 (1.3)	5/404 (1.2)
Other/Not reported, n/m (%)	0/18 (0.0)	0/16 (0.0)	6/433 (1.4)	NA
By Ethnicity				
Hispanic or Latino, n/m (%)	0/109 (0.0)	0/40 (0.0)	12/848 (1.4)	NA
Not Hispanic/Latino, n/m (%)	8/893 (0.9)	3/173 (1.7)	30/3331 (0.9)	2/57 (3.5)
Not Reported, n/m (%)	5/181 (2.8)	NA	19/931 (2.0)	3/352 (0.9)

Source: Adapted from Tables 4.4.13.1, 4.4.13.2, 4.4.13.3, 4.4.14.1, 4.4.14.2, 4.4.15.1, 4.4.15.2, 4.4.16.1 and 4.4.16.2, Response to Comment IR 10, Module 1.11.3, STN125678/0.14, and Response to IR 24, Module 1.11.3, STN125678/0.35.
Note: N=total number of subjects in the specified treatment group; m=total number of subjects in the specified subgroup; n=number of subjects with at least one reported SAE; NA=not applicable.

Reviewer's comment: *The total numbers of study subjects in the placebo group and the MVA-BN vaccinated vaccinia-naïve population group were 1183 and 5110, respectively. However, Tables 4.4.15.1 and 4.4.15.2 (STN125678/0.14) showed the total number of study subjects in the placebo group and the MVA-BN group in the subsets stratified by race were 1162 and 5062, respectively. An IR was sent on 14 May 2019 to the applicant for clarification. The applicant submitted its responses to STN125678/0.35. The correct information is reflected in Table 62.*

8.4.2.2 Nonfatal Serious Adverse Event in Pooled ISS Population (Including All 22 Studies)

Overview of SAEs in the Pooled ISS Population

An overview of the pooled analysis of SAEs from all the 22 clinical trials under the drug development program is provided in Table 63.

Among vaccinia naïve healthy subjects, the percentages of subjects who experienced any SAEs were numerically higher among MVA-BN vaccinated

subjects (1.4%) compared with placebo receipts (1.1%). The numerically higher percentage of subjects with any SAE among MVA-BN vaccinated subjects was likely attributed to the different study populations in the pooled ISS population between MVA-BN and placebo recipients. As shown in POX-MVA-013, the major randomized, placebo-controlled study which constituted of >80% placebo recipients in the pooled ISS, the percentage of subjects who reported any SAE was the same, 0.8%, for both MVA-BN and placebo recipients.

Among the MVA-BN vaccinated vaccinia naïve population, the percentage of subjects experiencing SAEs among AD subjects (1.3%) was similar to the healthy subjects (1.4%), while the percentage of subjects with any SAE was significantly higher in HIV infected subjects (4.2%) (Table 63). Similarly, among the MVA-BN vaccinated vaccinia-experienced population, the percentage of subjects with any SAE was numerically higher among HIV infected subjects (3.7%) compared with healthy subjects (1.5%) (Table 63).

Among all MVA-BN vaccinated subjects, the percentage of subjects with any SAE was numerically higher in vaccinia-experienced subjects (2.3%) compared with vaccinia-naïve population (1.5%). However, this was likely driven by the SAE cases in HIV-infected vaccinia-experienced subjects because the percentages of subjects with any SAE were similar between vaccinia-naïve healthy subjects (1.4%) and vaccinia-experienced healthy subjects (1.5%) (Table 63).

Table 63: Percentages of Subjects with Any Serious Adverse Event (SAE) Stratified by Treatment, Health Status and Previous Experience with Smallpox Vaccines (ISS Safety Population

Treatment/Population	Percentage of Subjects with Any SAE, % (95% CI)
Placebo/Vaccinia Naïve Subjects	
Healthy Subjects (N=1206, n=13)	1.1 (0.6, 1.8)
MVA-BN/Vaccinia Naïve Subjects	
All Vaccinia Naïve Subjects (N=7093, n=109)	1.5 (1.3, 1.9)
Healthy Subjects (N=6216, n=84)	1.4 (1.1, 1.7)
AD Subjects (N=381, n=5)	1.3 (0.4, 3.0)
HIV Infected Subjects (N=478, n=20)	4.2 (2.6, 6.4)
Others (N=18, n=0)	0.0 (0.0, 18.5)
ACAM2000/Vaccinia Naïve Subjects	
Healthy Subjects (N=213, n=3)	1.4 (0.3, 4.1)
MVA-BN/Vaccinia Experienced Subjects	
All Vaccinia Experienced Subjects (N=766, n=18)	2.3 (1.4, 3.7)
Healthy Subjects (N=532, n=8)	1.5 (1.3, 1.9)
HIV Infected Subjects (N=218, n=8)	3.7 (1.6, 7.1)

Source: Adapted from Tables 5.3.1.1 and 5.3.1.2, Response to IR 10_Q5-10, Module 1.11.3, STN125678/0.18

Note: AD=atopic dermatitis; N=number of subjects in the specified group; n=numbers of subjects with any SAE. Others=14 subjects with allergic rhinitis and 4 subjects with hematopoietic stem cell transplants.

The most commonly reported SAEs by SOC in MVA-BN treated subjects were Infections and Infestations, 0.4% (25 out of 7093 subjects) in vaccinia-naïve subjects and 0.8% (6 out of 766 subjects) in vaccinia-experienced subjects

(Table 64). No clustering pattern of SAEs by preferred term was observed among treatment group or subsets of the study populations. The majority of SAEs by preferred term was reported by one subject, and a few SAEs by preferred term were reported by two subjects. SAEs by preferred term that were reported by three or more MVA-BN treated subjects included appendicitis (by 5 vaccinia-naïve subjects), pneumonia (by 3 vaccinia-naïve and 1 vaccinia-experienced subjects), and depression, induced abortion, intentional overdose, spontaneous abortion (each by 3 vaccinia-naïve subjects), and prostate cancer (by 3 vaccinia-experienced subjects).

Table 64: Summary of Subjects with Any Serious Adverse Event (≥0.1%) by System Organ Class and Preferred Term (ISS Safety Population)

System Organ Class Preferred Term	Placebo Vaccinia Naïve Healthy Subjects	ACAM2000 Vaccinia Naïve Healthy Subjects	MVA-BN Vaccinia Naïve Subjects	MVA-BN Vaccinia Experienced Subjects
	(N=1206) n (%)	(N=213) n (%)	(N=7093) n (%)	(N=766) n (%)
Any	**13 (1.1)**	**3 (1.4)**	**109 (1.5)**	**18 (2.3)**
Blood and lymphatic system disorders	**0 (0.0)**	**0 (0.0)**	**4 (0.1)**	**0 (0.0)**
Gastrointestinal disorders	**0 (0.0)**	**1 (0.5)**	**15 (0.2)**	**2 (0.3)**
Diarrhea	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)
Hemorrhoids thrombosed	0 (0.0)	1(0.5)	0 (0.0)	0 (0.0)
Pancreatitis		0 (0.0)	2 (0.0)	1 (0.1)
General disorders and administration site conditions	**0 (0.0**)	**0 (0.0)**	**3 (0.0)**	**1 (0.1)**
Non-cardiac chest pain	0 (0.0)	0 (0.0)	1 (0.0)	1 (0.1)
Infections and infestations	**1 (0.1)**	**0 (0.0)**	**25 (0.4)**	**6 (0.8)**
Appendicitis	0 (0.0)	0 (0.0)	5 (0.1)	0 (0.0)
Cellulitis	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)
Gangrene	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)
Gastroenteritis	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.4)
Gastroenteritis salmonella	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Pneumonia	0 (0.0)	0 (0.0)	3 (0.0)	1 (0.1)
Injury, poisoning and procedural complications	**5 (0.4)**	**1 (0.5)**	**20 (0.3)**	**3 (0.4)**
Ankle fracture	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Concussion	0 (0.0)	0 (0.0)	2 (0.0)	1 (0.1)
Lower limb fracture	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)
Maternal exposure before pregnancy	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Pelvic fracture	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Road traffic accident	0 (0.0)	1 (0.5)	0 (0.0)	1 (0.1)
Tendon rupture	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)
Skin abrasion	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Investigations	**1 (0.1)**	**0 (0.0)**	**0 (0.0)**	**1 (0.1)**
ECG abnormal	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Platelet count increased	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)
Musculoskeletal and connective tissue disorders	**0 (0.0)**	**1 (0.5)**	**3 (0.0)**	**0 (0.0)**
Rhabdomyolysis	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)
Neoplasms benign, malignant and unspecified	**2 (0.2)**	**0 (0.0)**	5 (0.1)	**3 (0.4)**
Benign neoplasm of thyroid gland	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Colon cancer	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Prostate cancer	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.4)
Nervous system disorders	**2 (0.2)**	**0 (0.0)**	**7 (0.1)**	**0 (0.0)**
Neurological symptom	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Seizure	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Pregnancy, puerperium and perinatal conditions	**2 (0.2)**	**0 (0.0)**	**5 (0.1)**	**0 (0.0)**
Abortion spontaneous	2 (0.2)	0 (0.0)	3 (0.0)	0 (0.0)
Psychiatric disorders	**2 (0.2)**	**0 (0.0)**	**11 (0.1)**	**0 (0.0)**
Depression	2 (0.2)	0 (0.0)	3 (0.0)	
Suicide attempt	1 (0.1)	0 (0.0)	2 (0.0)	
Respiratory, thoracic and mediastinal disorders	**0 (0.0)**	**0 (0.0)**	**4 (0.1)**	**1 (0.1)**
Pneumothorax	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)
Surgical and medical procedures	**0 (0.0)**	**0 (0.0)**	**7 (0.1)**	**0 (0.0)**
Vascular disorders	**0 (0.0)**	**0 (0.0)**	**2 (0.0)**	**1 (0.1)**
Peripheral arterial occlusive disease	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)

Source: Adapted from Tables 5.3.1.1 and 5.3.1.2, Response to IR 10_Q5-10, Module 1.11.3, STN125678/0.18

Note: N=number of subjects in specific group; n=number of subjects with specific event.

MVA-BN Related SAEs

A total of 7 SAEs reported by 7 subjects following MVA-BN vaccination were assessed as at least possibly related to MVA-BN by the study investigators. A summary of these cases is presented in Table 65.

Table 65: Summary of Serious Adverse Events that Were Assessed as Related to Treatment by Investigators (ISS Population)

Subject ID	Age/Sex	SAE	SAE Onset Related to Treatment	Causality	Outcome
POX-MVA-005					
(b) (6)	30/Male	Sarcoidosis	10 weeks after the 2nd dose	Possibly related	Ongoing
(b) (6)	28/Female	Crohn's disease	26 months after the 2nd dose	Possibly related	Asymptomatic
POX-MVA-008					
(b) (6)	28/Female	Extraocular muscle paresis	8 days after the 2nd dose	Possibly related	Resolved
POX-MVA-010					
(b) (6)	30/Female	Cardiomyopathy	133 days after the 2nd dose	Possibly related	Ongoing
POX-MVA-011					
(b) (6)	39/Female	Pneumonia	The day after the 2nd dose	Possibly related	Resolved
POX-MVA-036					
(b) (6)	30/Male	Non-ST elevation myocardial infarction	4 months after a single dose	Possibly related	Resolved
(b) (6)	27/Female	Hypersensitivity	2 hours after the 2nd dose	Possibly related	Resolved

Source: Summarized from page 27-40, ISS Section 8_Narratives, Module 5.3.5.3, STN125678/0.

Narratives of MVA-BN Related SAEs

SAEs that were assessed at least possibly related to study treatment are briefly summarized below.

Sarcoidosis (POX-MVA-005): Please refer to Section 6.2.12.4.

Crohn's disease (POX-MVA-005): This case did not occur during the study period of POX-MVA-005 but was identified at 2 years after the last MVA-BN vaccination during screening for study POX-MVA-023. Due to a significantly elevated alkaline phosphatase as well as elevated absolute neutrophil and platelet counts at screening for the POX-MVA-023 trial, the study investigator suggested the otherwise asymptomatic subject consult her primary care physician who subsequently recommended an endoscopy be performed. The subject was diagnosed with Crohn's disease and the applicant was informed of the diagnosis on 16 September 2009. The subject had been asymptomatic. Since there was no alternative etiology, the investigator considered the event possibly related to the treatment, while the applicant considered it unlikely related

to MVA-BN but stated that a causal relationship could not be completely ruled out.

Reviewer's comment: *The reported Crohn's disease does not appear to meet the criteria for an SAE because the subject had been asymptomatic. Nevertheless, this reviewer concurs with the applicant that the event appeared to be unlikely related to the treatment, however, a causal relationship with MVA-BN could not be ruled out.*

Extraocular muscle paresis (POX-MVA-008): A 28-year-old, healthy, vaccinia-naïve female subject received the first vaccination with MVA-BN on (b) (6) and the second vaccination on (b) (6) . She experienced extraocular muscle paresis 8 days after the second vaccination. The subject experienced constant mixed horizontal and vertical diplopia. Upon examination by an ophthalmologist on (b) (6) , the subject was diagnosed with a right lower oculomotor muscle paresis. On (b) (6) , her right hypertropia was considered to be improving and diplopia was less bothersome. On (b) (6) , the subject presented with a red eye and increased palpebral volume. She was diagnosed with bacterial conjunctivitis. By (b) (6) the paresis had almost completely recovered and neither diplopia nor hypertropia were evident. By (b) (6) , the diplopia had completely disappeared, and the conjunctivitis was resolved.

In the absence of other risk factors, the attending neurologist and the applicant considered the event to be a possible vaccination adverse reaction.

Reviewer's comment:
No other cases of any type of paralysis or similar events have been reported in this submission or following administration of MVA based products to this reviewer's knowledge. This reviewer considers this event unlikely related to the vaccination. However, this reviewer agrees that a causal relationship could not be completely ruled out due to temporal association and lack of alternative etiology.

Congestive heart failure due to cardiomyopathy (POX-MVA-010): A 30-year-old, African American, HIV-infected, vaccinia-naïve female subject was hospitalized 133 days following her last MVA-BN vaccination due to congestive heart failure. Clinical signs, symptoms and medical history included shortness of breath, pleural effusion, hypertension, obesity, dyspnea on exertion, glaucoma, and osteopenia. She was diagnosed with congestive heart failure due to cardiomyopathy and was discharged from the hospital after 10 days in stable condition with cardiac medications.

The subject had been concurrently participating in a growth hormone releasing hormone (GH-RH) study for treatment of lipodystrophy; she did not report participating in another study during screening for the MVA trial, otherwise this would have excluded her participation. The lipodystrophy study investigator also

assessed the event “congestive cardiac failure” as possibly related to the study drug GH-RH.

The applicant assessed the event to be unlikely related to MVA-BN. However, the applicant also stated that a causal relationship could not be entirely ruled out.

Reviewer’s Comment*: HIV infection is associated with cardiomyopathy in 10-15% of cases[16]. In the presence of HIV infection, lipodystrophy and concomitant treatment with GH-RH, it is difficult to assess the causality of the reported cardiomyopathy. This reviewer agrees with the applicant that the event was unlikely caused by MVA-BN.*

Pneumonia (POX-MVA-011): A 39-year-old HIV infected, vaccinia-naïve white female was hospitalized with pneumonia the day after the second vaccination on (b) (6) . Based on two view chest x-rays performed on the day of hospital admission, mild subsegmental atelectasis was suspected within the right lower lung, but otherwise the views of the chest were unremarkable. The subject was treated with antibiotics for pneumonia and was released home on (b) (6) . She recovered without sequelae and continued the study as planned. The subject had a history of chronic obstructive pulmonary disease (COPD). The Investigator and the applicant assessed the pneumonia as possibly related to study medication due to the temporal association.

On (b) (6) , the subject was hospitalized again with pneumonia and was treated with oxygen, IV fluid and antibiotics. She was discharged on (b) (6) .

Reviewer’s comment: *Bacterial pneumonia was likely attributed to the subject’s underlying medical condition. There is no biological plausibility that virus based vaccines could cause bacterial pneumonia. This reviewer considers that the pneumonia was unlikely related to the vaccination.*

Hypersensitivity (POX-MVA-036): A healthy, vaccinia-naïve female subject experienced itching all over, skin rash and throat tightness 2 hours after she received her second dose of MVA-BN. She came to the investigator and had obvious hives on her chest, arms and neck, red and swollen ears, angioedema of her forearms, and throat tightness. She was taken to the emergency room and treated with Benadryl and epinephrine. Her symptoms improved significantly, and she was discharged on the same day. Symptoms subsided after several days on prednisone and diphenhydramine treatment. She had a family history of allergies and a medical history of shingles. She had received multiple vaccines before but never had previous hives or other problems with vaccines. Both investigator and the applicant considered the event as possible anaphylaxis and possibly related to treatment.

Reviewer’s comment: *Due to the close temporal association of the event with the treatment, the absence of alternative etiology, and the fact that*

hypersensitivity reaction occurred shortly after the second dose of MVA-BN, this reviewer considered the SAE probably related to MVA-BN.

Non-ST segment elevation myocardial infarction (MI) (POX-MVA-036): Please refer to Section 8.4.8 for narrative of this case (Subject (b) (6)).

Reviewer's comment: *This reviewer has also assessed all other SAEs reported in this submission and concurs with the applicant's causality assessments.*

Subgroup Analyses of SAEs

Subgroup analyses of SAEs stratified by age, sex, race and ethnicity are presented in Table 66. The results are similar to those in the Main ISS.

Table 66: Subgroup Analyses of Serious Adverse Events (SAEs) in Pooled ISS Population (Safety Population)

Subgroup	**Placebo** Vaccinia Naïve Healthy Subjects	**ACAM2000** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Naïve Subjects	**MVA-BN** Vaccinia Experienced Subjects
	Placebo (N=1206)	ACAM2000 (N=213)	MVA-BN (N=7093)	MVA-BN (N=766)
Any, n/N (%)	**13/1206 (1.1)**	**3/213 (1.4)**	**109/7093 (1.5)**	**18/766 (2.3)**
By Age (Years)				
18-40, n/m (%)	12/1201 (1.0)	3/212 (1.4)	104/6902 (1.5)	NA
>40, n/m (%)	1/5 (20.0)	0/1 (0.0)	5/191 (2.6)	NA
18-55, n/m (%)	NA	NA	NA	12/640 (1.9)
>55, n/m (%)	NA	NA	NA	6/126 (4.8)
By Sex				
Male, n/m (%)	5/552 (0.9)	3/184 (1.6)	50/3613 (1.4)	12/417 (2.9)
Female, n/m (%)	8/654 (1.2)	0/29 (0.0)	59/3480 (1.7)	6/349 (1.7)
By Race				
American Indian or Alaska Native, n/m (%)	1/7 (14.3)	0/6 (0.0)	0/25 (0.0)	0/0 (0.0)
Asian, n/m (%)	0/21 (0.0)	0/12 (0.0)	1/225 (0.4)	0/6 (0.0)
Black, n/m (%)	1/186 (0.5)	1/40 (2.5)	14/1125 (1.2)	5/82 (6.1)
Native Hawaiian or Other Pacific Islander, n/m (%)	0/3 (0.0)	0/3 (0.0)	0/26 (0.0)	0/0 (0.0)
White, n/m (%)	11/971 (1.1)	2/136 (1.5)	87/5196 (1.7)	13/649 (2.0)
Other/Not reported, n/m (%)	0/18 (0.0)	0/16 (0.0)	7/496 (1.4)	0/29 (0.0)
By Ethnicity				
Hispanic or Latino, n/m (%)	0/110 (0.0)	0/40 (0.0)	15/1012 (1.5)	1/29 (3.4)
Not Hispanic/Latino, n/m (%)	8/915 (0.9)	3/173 (1.7)	59/4608 (1.3)	5/138 (3.6)
Not Reported, n/m (%)	5/181 (2.8)	0/0 (0.0)	35/1473 (2.4)	12/599 (2.0)

Source: Adapted from Tables 5.3.2.1, 5.3.2.2, 5.3.3.1, 5.3.3.2, 5.3.4.1, 5.3.4.2, 5.3.5.1, and 5.3.5.2, Response to Comment RFI 10-Q5-10, Module 1.11.3, STN125678/0.18.
Note: N=total number of subjects in the specified treatment group; m=total number of subjects in the specified subgroup; n=number of subjects with at least one reported SAE; NA=not applicable.

8.4.3 Study Dropouts/Discontinuations

Study subjects who dropped out or discontinued from the studies and the corresponding reasons for withdrawal in the pooled Main ISS population are summarized in Table 67. The most common reason for discontinuation was non-

compliance with treatment or follow-up (1.3% to 4.9%) followed by other non-specified reasons (1.1% to 2.5%).

Twenty-two subjects (0.4%) in the vaccinia-naïve population discontinued study due to AE, which was comparable to the placebo control group (0.3%). No subject among the vaccinia experienced population discontinued study due to AE.

No particular pattern regarding the nature of the AEs leading to withdrawal was identified. Most AEs were in the SOCs of Investigations (5 healthy, vaccinia-naïve subjects) and General Disorders and Administration Site Conditions (2 healthy, vaccinia-naïve subjects and 1 HIV infected, vaccinia-naïve subject).

Of note, 3 subjects (all healthy, vaccinia-naïve) withdrew from the second vaccination due to cardiac disorders (right bundle branch block, pericarditis, and Wolff-Parkinson-White syndrome); and 1 subject each (also healthy, vaccinia-naïve subjects) withdrew due to an abnormal ECG, elevated troponin I, and chest pain, which translated to 0.1% of subjects who discontinued from further vaccination because of cardiac related events.

Table 67: Summary of Subjects with Study Discontinuation (Main ISS)

Characteristics	**MVA-BN** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Naïve AD Subjects	**MVA-BN** Vaccinia Naïve HIV Subjects	**MVA-BN** Total Vaccinia Naïve Subjects	**Placebo** Vaccinia Naïve Healthy Subjects	**ACAM2000** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Experienced Healthy Subjects
	(N=4381) n (%)	(N=350) n (%)	(N=379) n (%)	(N=5110) n (%)	(N=1183) n (%)	(N=213) n (%)	(N=409) n (%)
Study Completion							
Completed	4087 (93.3%)	325 (92.9%)	350 (92.3%)	4762 (93.2%)	1121 (94.8%)	204 (95.8%)	408 (99.8%)
Discontinued	294 (6.7%)	25 (7.1%)	29 (7.7%)	348 (6.8%)	62 (5.2%)	9 (4.2%)	1 (0.2%)
Reasons for Discontinuation							
Adverse Event	22 (0.5%)	0 (0.0%)	0 (0.0%)	22 (0.4%)	4 (0.3%)	0 (0.0%)	0 (0.0%)
Withdrawal by Subject	39 (0.9%)	2 (0.6%)	5 (1.3%)	46 (0.9%)	11 (0.9%)	1 (0.5%)	0 (0.0%)
Non-Compliance	94 (2.1%)	17 (4.9%)	8 (2.1%)	119 (2.3%)	15 (1.3%)	4 (1.9%)	1 (0.2%)
Therapy not Permitted	1 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Lost to Follow-up	23 (0.5%)	0 (0.0%)	0 (0.0%)	23 (0.5%)	1 (0.1%)	0 (0.0%)	0 (0.0%)
Physician Decision	4 (0.1%)	0 (0.0%)	0 (0.0%)	4 (0.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Pregnancy	6 (0.1%)	2 (0.6%)	0 (0.0%)	8 (0.2%)	2 (0.2%)	0 (0.0%)	0 (0.0%)
Protocol Deviation	3 (0.1%)	0 (0.0%)	0 (0.0%)	3 (0.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Unrelated Illness or Injury	0 (0.0%)	0 (0.0%)	1 (0.3%)	1 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Other	102 (2.3%)	4 (1.1%)	15 (4.0%)	121 (2.4%)	29 (2.5%)	4 (1.9%)	0 (0.0%)

Source: Adapted from Tables 1.2.1 and 1.2.2, Appendix 1 to Main ISS, Module 5.3.5.3_Main ISS, STN125678/0.7.

8.4.4 Common Adverse Events

8.4.4.1 Overview of Unsolicited Adverse Events

The unsolicited adverse events reported by subjects in the Main ISS population are summarized in Table 68. Overall a greater proportion of MVA-BN vaccinated vaccinia-naïve subjects (37.9%) and vaccinia-experienced subjects (41.6%) reported at least one unsolicited AE compared to subjects who received placebo (23.2%), but lower than ACAM200 vaccinated subjects (97.7%). Among the MVA-BN vaccinated vaccinia-naïve subjects, a greater proportion of subjects reported unsolicited AEs in the AD subjects (59.1%) and HIV infected subjects (60.2%) as compared with healthy subjects (34.3%).

MVA-BN vaccinated subjects reported more severe (grade 3) unsolicited AEs (2.5 % for vaccinia-naïve subjects and 2.7% for vaccinia experienced subjects) compared with placebo recipients (1.0%), but fewer compared with subjects vaccinated with ACAM2000 (8.5%). Among MVA-BN vaccinated vaccinia-naïve subjects, AD subjects and HIV infected subjects reported more severe unsolicited AEs, 4.0% and 7.4% respectively (Table 68).

A greater proportion of MVA-BN vaccinated subjects (15.9%) reported unsolicited AEs that were at least possibly related to treatment as assessed by investigators compared with placebo recipients (4.4%), but less than ACAM2000 vaccinated subjects (96.2%) (Table 68). Among MVA-BN vaccinated vaccinia-naïve subjects, AD subjects (32.6%) and HIV infected subjects (21.4%) reported more treatment related unsolicited AEs compared with healthy subjects (14.2%) (Table 68).

Table 68: Summary of Unsolicited Adverse Events – Main ISS (Safety Population)

AE	**MVA-BN** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Naïve AD Subjects	**MVA-BN** Vaccinia Naïve HIV Subjects	**MVA-BN** Total Vaccinia Naïve Subjects	**Placebo** Vaccinia Naïve Healthy Subjects	**ACAM2000** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Experienced Healthy Subjects
	(N=4381) n (%)	(N=350) n (%)	(N=379) n (%)	(N=5110) n (%)	(N=1183) n (%)	(N=213) n (%)	(N=409) n (%)
Any AE	1502 (34.3)	207 (59.1)	228 (60.2)	1937 (37.9)	274 (23.2)	208 (97.7)	170 (41.6)
Related AE	620 (14.2)	114 (32.6)	81 (21.4)	815 (15.9)	52 (4.4)	205 (96.2)	73 (17.8)
Severe AE	84 (1.9)	14 (4.0)	28 (7.4)	126 (2.5)	12(1.0)	18 (8.5)	11 (2.7)
Related Severe AE	14 (0.3)	2 (0.6)	6 (1.6)	22 (0.4)	2 (0.2)	11 (5.2)	2 (0.5)

Source: Adapted from Tables 4.1.1.1, 4.1.1.2, 4.1.4.1, 4.1.4.2, 4.1.5.1, 4.1.5.2, 4.3.2.1 and 4.3.2.2, Module 5.3.5.3, STN125678/0.
Note: N=total number of subjects in the specified treatment group; n=number of subjects with at least one reported unsolicited adverse event.

The unsolicited adverse events that occurred in ≥1% subjects stratified by healthy status, previous smallpox vaccination status, SOC and PT are presented in Table 69. The overall proportion of subjects who experienced unsolicited AEs was comparable between vaccinia-naïve subjects and vaccinia-experienced subjects. The proportion of subjects reporting unsolicited AEs was slightly higher among subjects with AD and HIV-infection. Among MVA-BN vaccinated subjects, the most commonly reported unsolicited AEs by PT were upper respiratory tract infection (2.9%), viral upper respiratory tract infection (2.8%), injection-site induration (2.7%), and troponin increase (2.6%).

No pattern or cluster in unsolicited AEs is observed among MVA-BN vaccinated subjects.

Reviewer's comment: *The higher proportion of subjects with abnormal troponin was driven by studies POX-MVA-008 and POX-MVA-011 in which approximately 11-18% of subjects experienced increased troponin post MVA-BN vaccination. Please refer to Section 8.4.8 for further discussion of this issue.*

For solicited adverse events, please refer to Sections 8.4.6 and 8.4.7.

Table 69: Unsolicited Adverse Events Occurred in ≥1% Subjects by System Organ Class and Preferred Term – Main ISS (Safety Population)

System Organ Class Preferred Term	**MVA-BN** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Naïve AD Subjects	**MVA-BN** Vaccinia Naïve HIV Subjects	**MVA-BN** Total Vaccinia Naïve Subjects	**Placebo** Vaccinia Naïve Healthy Subjects	**ACAM2000** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Experienced Healthy Subjects
	(N=4381) n (%)	(N=350) n (%)	(N=379) n (%)	(N=5110) n (%)	(N=1183) n (%)	(N=213) n (%)	(N=409) n (%)
Blood and lymphatic system disorders	**50 (1.1)**	**2 (0.6)**	**4 (1.1)**	**56 (1.1)**	**3 (0.3)**	**109 (51.2)**	**6 (1.5)**
Lymphadenopathy	50 (1.1)	2 (0.6)	4 (1.1)	56 (1.1)	3 (0.3)	109 (51.2)	6 (1.5)
Gastrointestinal disorders	**39 (0.9)**	**9 (2.6)**	**15 (4.0)**	**63 (1.2)**	**0 (0.0)**	**0 (0.0)**	**11 (2.7)**
Diarrhea	39 (0.9)	9 (2.6)	15 (4.0)	63 (1.2)			11 (2.7)
General disorders and administration site conditions	**284 (6.5)**	**38 (10.9)**	**15 (4.0)**	**337 (6.6)**	**0 (0.0)**	**69 (32.4)**	**25 (6.1)**
Injection site induration	135 (3.1)	1 (0.3)	1 (0.3)	137 (2.7)		1 (0.5)	1 (0.2)
Injection site nodule	95 (2.2)	4 (1.1)	6 (1.6)	105 (2.1)		2 (0.9)	2 (0.5)
Injection site pruritus	19 (0.4)	30 (8.6)	8 (2.1)	57 (1.1)		0 (0.0)	4 (1.0)
Injection site warmth	37 (0.8)	6 (1.7)	2 (0.5)	45 (0.9)		0 (0.0)	14 (3.4)
Malaise	40 (0.9)	0 (0.0)	2 (0.5)	42 (0.8)		66 (31.0)	1 (0.2)
Infections and infestations	**253 (5.8)**	**23 (6.6)**	**15 (4.0)**	**291 (5.7)**	**64 (5.4)**	**14 (6.6)**	**35 (8.6)**
Upper respiratory tract infection	137 (3.1)	6 (1.7)	7 (1.8)	150 (2.9)	32 (2.7)	14 (6.6)	1 (0.2)
Viral upper respiratory tract infection	117 (2.7)	17 (4.9)	8 (2.1)	142 (2.8)	32 (2.7)	0 (0.0)	34 (8.3)
Investigations	**50 (1.1)**	**51 (14.6)**	**33 (8.7)**	**134 (2.6)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**
Troponin I increased	50 (1.1)	51 (14.6)	33 (8.7)	134 (2.6)			
Musculoskeletal and connective tissue disorders	**48 (1.1)**	**0 (0.0)**	**5 (1.3)**	**53 (1.0)**	**0 (0.0)**	**0 (0.0)**	**3 (0.7)**
Arthralgia	48 (1.1)		5 (1.3)	53 (1.0)			3 (0.7)
Nervous system disorders	**92 (2.1)**	**25 (7.1)**	**18 (4.7)**	**135 (2.6)**	**0 (0.0)**	**0 (0.0)**	**24 (5.9)**
Headache	62 (1.4)	15 (4.3)	9 (2.4)	86 (1.7)			18 (4.4)
Dizziness	33 (0.8)	11 (3.1)	12 (3.2)	56 (1.1)			6 (1.5)
Respiratory, thoracic and mediastinal disorders	**90 (2.1)**	**4 (1.1)**	**28 (7.4)**	**122 (2.4)**	**17 (1.4)**	**1 (0.5)**	**9 (2.2)**
Oropharyngeal pain	58 (1.3)	2 (0.6)	15 (4.0)	75 (1.5)	17 (1.4)	1 (0.5)	8 (2.0)
Cough	34 (0.8)	3 (0.9)	17 (4.5)	54 (1.1)	0 (0.0)	0 (0.0)	1 (0.2)

Source: Adapted from Tables 4.1.6.1, 4.1.6.2, 4.1.7.1 and 4.1.7.2, Response to Comments RFI 10, Module 1.11.3, STN125678/0.14.
Note: AD=atopic dermatitis; N=number of subjects in the specified group; n=number subjects with the specified AE; %=n/N X100.

8.4.4.2 Subgroup Analysis of Unsolicited Adverse Events

Analyses of subgroups with regard to proportions of subjects reporting any unsolicited adverse events, stratified by age, sex, race and ethnicity, are presented in Table 70.

Among vaccinia naïve subjects vaccinated with MVA-BN, stratification by age, a slightly higher incidence of unsolicited AEs was reported among the subjects >40 years of age subgroup. The Non-Hispanic/Latino subgroup showed lower incidence of unsolicited AEs compared with Hispanic/Latino subgroup or the subgroup with not-reported ethnicity. There was no meaningful difference in percentage of subjects with any unsolicited AEs among the subgroups stratified by sex and race.

Among vaccinia experienced subjects vaccinated with MVA-BN, the proportion of subjects with unsolicited AEs was similar among subgroups stratified by age and ethnicity. Stratification by sex showed that females had a slightly higher incidence of unsolicited AEs compared with males. Given that 98.8% (404 out of 409) of subjects were white, subgroup analysis stratified by race is unlikely to provide clinically relevant data due to the small numbers of subjects in the non-white population.

Table 70: Subgroup Analyses of Unsolicited Adverse Events in Pooled Main ISS Population (Safety Population)

Subgroup	**Placebo** Vaccinia Naïve Healthy Subjects	**ACAM200** Vaccinia Naïve Healthy Subjects	**MVA-BN** All Vaccinia Naïve Subjects	**MVA-BN** All Vaccinia Experienced Healthy Subjects
	(N=1183)	(N=213)	(N=5110)	MVA-BN (N=409)
Any, n/N (%)	**273/1183 (23.2)**	**208/213 (97.7)**	**1937/5110 (37.9)**	**170/409 (41.6)**
By Age (Years)				
18-40, n/m (%)	270/1178 (22.9)	207/212 (97.6)	1859/4963 (37.5)	NA
>40, n/m (%)	4/5 (80.0)	1/1 (100.0)	78/147 (53.1)	NA
18-55, n/m (%)	NA	NA	NA	146/352 (41.5)
>55, n/m (%)	NA	NA	NA	24/57 (42.1)
By Sex				
Male, n/m (%)	111/537 (20.7)	179/184 (97.3)	945/2618 (36.1)	61/169 (36.1)
Female, n/m (%)	163/646 (25.2)	29/29 (100.0)	992/2492 (39.8)	109/240 (45.4)
By Race				
American Indian or Alaska Native, n/m (%)	NA	6/6 (100.0)	9/24 (37.5)	NA
Asian, n/m (%)	8/20 (40.0)	12/12 (100.0)	60/166 (36.1)	1/3 (33.3)
Black, n/m (%)	27/184 (14.7)	39/40 (97.5)	280/883 (31.7)	2/2 (100.0)
Native Hawaiian or Other Pacific Islander, n/m (%)	1/3 (33.3)	3/3 (100.0)	10/21 (47.6)	0/0 (0.0)
White, n/m (%)	235/951 (24.7)	132/136 (97.1)	1322/3583 (36.9)	167/404 (41.3)
Other/Not reported, n/m (%)	3/18 (16.7)	16/16/ (100.0)	254/430 (59.1)	0/0 (0.0)
By Ethnicity				
Hispanic or Latino, n/m (%)	24/109 (22.0)	38/40 (95.0)	405/848 (47.8)	0/0 (0.0)
Not Hispanic/Latino, n/m (%)	173.893 (19.4)	170/173 (98.3)	988/3331 (29.7)	24/57 (42.1)
Not Reported, n/m (%)	77/181 (42.5)	0/0 (0.0)	544/931 (58.4)	146/352 (41.5)

Source: Adapted from Tables 4.8.1.1, 4.8.1.2, 4.8.1.3, 4.8.2.1, 4.8.2.2, 4.8.3.1, 4.8.3.2, 4.8.4.1, and 4.8.4.2, Response to Comment RFI 10, Module 1.11.3, STN125678/0.14.
Note: N=total number of subjects in the specified treatment group; m=total number of subjects in the specified subgroup; n=number of subjects with at least one AE; NA=not applicable.

8.4.5 Clinical Test Results

No clinically meaningful abnormal laboratory results were reported in the clinical studies except for troponin. Please refer to Section 8.4.8.2 for troponin analysis.

8.4.6 Solicited Systemic Adverse Reactions

8.4.6.1 Overview of Solicited Systemic Adverse Reactions

An overview of the pooled solicited systemic reactions among the Main ISS population is provided in Table 71. The overall proportion of subjects who experienced any solicited adverse reactions was higher among MVA-BN recipients than placebo recipients (53.0%). Among the MVA-BN recipients, the proportion of subjects who experienced any solicited adverse reaction was slightly lower for vaccinia-naïve subjects (including healthy subjects, AD subjects and HIV infected subjects) compared with vaccinia-experienced subjects (88.8% versus 95.6%).

The proportions of MVA-BN recipients who experienced any solicited systemic adverse reaction (58.0% for vaccinia naïve subjects and 51.1% for vaccinia-experienced subjects) were similar compared with ACAM2000 recipients (57.7%), but higher than the placebo recipients (39.1%) (Table 71). Among the MVA-BN recipients, the proportions of subjects who experienced any solicited systemic reaction for vaccinia-naïve healthy subjects, AD subjects, and HIV infected subjects were 57.5%, 67.1%, and 55.1%, respectively. AD subjects and HIV infected subjects tended to have more grade 3 solicited system adverse reactions compared with vaccinia naïve healthy subjects (11.1% for AD, 7.1% for HIV and 5.6% for healthy subjects).

The most commonly reported solicited systemic adverse reactions were myalgia, headache and fatigue among MVA-BN recipients as well as placebo and ACAM2000 recipients (Table 71).

Table 71: Overview of Pooled Solicited Systemic Adverse Reactions – Main ISS (Safety Population)

Category	**MVA-BN** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Naïve AD Subjects	**MVA-BN** Vaccinia Naïve HIV Subjects	**MVA-BN** Total Vaccinia Naïve Subjects	**Placebo** Vaccinia Naïve Healthy Subjects	**ACAM2000** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Experienced Healthy Subjects
	(N=4381) n (%)	(N=350) n (%)	(N=379) n (%)	(N=5110) n (%)	(N=1183) n (%)	(N=213) n (%)	(N=409) n (%)
Any Solicited Reaction	**3913 (89.3)**	**312 (89.1)**	**312 (82.3)**	**4537 (88.8)**	**627 (53.0)**	**197 (92.5)**	**391 (95.6)**
≥Grade 3 Reaction	646 (14.7)	82 (23.4)	52 (13.7)	780 (15.3)	50 (4.2)	58 (27.2)	27 (6.6)
Any Systemic Reaction	**2518 (57.5)**	**235 (67.1)**	**209 (55.1)**	**2962 (58.0)**	**462 (39.1)**	**123 (57.7)**	**209 (51.1)**
≥Grade 3 Reaction	246 (5.6)	39 (11.1)	28 (7.4)	313 (6.1)	41 (3.5)	21 (9.9)	11 (2.7)
Any Myalgia	1637 (37.4)	153 (43.7)	131 (34.6)	1921 (37.6)	192 (16.2)	76 (35.7)	88 (21.5)
≥Grade 3 Myalgia	91 (2.1)	14 (4.0)	12 (3.2)	117 (2.3)	7 (0.6)	8 (3.8)	3 (0.7)
Any Headache	1517 (34.6)	163 (46.6)	127 (33.5)	1807 (35.4)	300 (25.4)	77 (36.2)	113 (27.6)
≥Grade 3 Headache	101 (2.3)	26 (7.4)	12 (3.2)	139 (2.7)	23 (1.9)	10 (4.7)	5 (1.2)
Any Fatigue	1350 (30.8)	125 (35.7)	117 (30.9)	1592 (31.2)	256 (21.6)	88 (41.3)	137 (33.5)
≥Grade 3 Fatigue	119 (2.7)	16 (4.6)	13 (3.4)	148 (2.9)	16 (1.4)	9 (4.2)	5 (1.2)
Any Nausea	711 (16.2)	80 (22.9)	59 (15.6)	850 (16.6)	141 (11.9)	41 (19.2)	40 (9.8)
≥Grade 3 Nausea	62 (1.4)	8 (2.3)	7 (1.8)	77 (1.5)	13 (1.1)	7 (3.3)	0 (0.0)
Any Chills	415 (9.5)	55 (15.7)	65 (17.2)	535 (10.5)	57 (4.8)	34 (16.0)	3 (0.7)
≥Grade 3 Chills	37 (0.8)	7 (2.0)	4 (1.1)	48 (0.9)	3 (0.3)	3 (1.4)	0 (0.0)
Any Pyrexia	96 (2.2)	7 (2.0)	9 (2.4)	112 (2.2)	11 (0.9)	2 (0.9)	2 (0.5)

Source: Adapted from Tables 10 (page 38-39) and 11 (page 41), Integrated Summary of Safety, Module 5.3.5.3-Main ISS, and Tables 3.3.1 and 3.3.2 (Appendix 1), STN125678/0.7.
Note: AD=atopic dermatitis; N=number of subjects in the specified group; n=number subjects with the specified AE; %=n/N X100.

8.4.6.2 Subgroup Analyses of Solicited Systemic Adverse Reactions

Subpopulation analyses of solicited systemic reactions based on the safety population of pooled Main ISS are presented in Table 72.

Among the vaccinia naïve subjects vaccinated with MVA-BN, percentages of subjects with solicited systemic adverse reactions were similar between different age (18 to 40 years of age vs. >40 years of age), and ethnicity (Hispanic/Latino vs. Non-Hispanic/Latino vs. others) groups. More female subjects (64.4%) reported solicited systemic adverse reactions compared with male subjects (51.8%). Percentages of subjects with solicited systemic reactions among American Indian/Alaska Native (62.5%), white (60.2%) and other/not reported (63.7%) subjects were numerically higher than those among Asian (45.8%), black/African (48.6%) and Native Hawaiian or Other Pacific Islander (47.6%) subjects.

Among vaccinia-experienced subjects vaccinated with MVA-BN, 98.8% (404 out 409) subjects were white and none were Hispanic/Latino. More female subjects (54.6%) experienced solicited systemic reactions compared with male subjects (46.2%), and more subjects >55 years (89.5%) reported solicited systemic adverse reactions compared with subjects 18 to 55 years of age (52.6%).

Table 72: Subgroup Analyses of Solicited Systemic Adverse Reactions – Main ISS (Safety Population)

Subgroup	**MVA-BN** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Naïve AD Subjects	**MVA-BN** Vaccinia Naïve HIV Subjects	**MVA-BN** Total Vaccinia Naïve Subjects	**Placebo** Vaccinia Naïve Healthy Subjects	**ACAM2000** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Experienced Healthy Subjects
	(N=4381) n/m (%)	(N=350) n/m (%)	(N=379) n/m (%)	(N=5110) n/m (%)	(N=1183) n/m (%)	(N=213) n/m (%)	(N=409) n/m (%)
Age							
18-40 years of age	2500/4353 (57.4)	234/349 (67.0)	144/261 (55.2)	2878/4963 (58.0)	461/1178 (39.1)	122/212 (57.5)	NA
>40 years of age	18/28 (64.3)	1/1 (100.0)	65/118 (55.1)	84/147 (57.1)	1/20 (20.0)	1/1 (100.0)	NA
18-55 years of age	NA	NA	NA	NA	NA	NA	185/352 (52.6)
>55 years of age	NA	NA	NA	NA	NA	NA	51/57 (89.5)
Sex							
Male	1126/2180 (51.7)	60/127 (47.2)	170/311 (54.7)	1356/2618 (51.8)	175/537 (32.6)	100/184 (54.3)	78/169 (46.2)
Female	1392/2201 (63.2)	175/223 (78.5)	39/68 (57.4)	1606/2492 (64.4)	287/646 (44.4)	23/29 (79.3)	131/240 (54.6)
Race							
American Indian or Alaska Native	14/23 (60.9)	NA	1/1 (100.0)	15/24 (62.5)	3/7 (42.9)	4/6 (66.7)	NA
Asian	56/113 (49.6)	19/51 (37.3)	1/2 (50.0)	76/166 (45.8)	9/20 (45.0)	8/12 (66.7)	2/3 (66.7)
Black/African American	353/715 (49.4)	23/33 (69.7)	53/135 (39.3)	429/883 (48.6)	49/184 (26.6)	21/40 (52.5)	2/2 (100.0)
Native Hawaiian or Other Pacific Islander	8/19 (42.1)	1/1 (100.0)	1/1 (100.0)	10/21 (47.6)	0 /3(0.0)	1/3 (33.3)	NA
White	1954/3285 (59.5)	91/127 (71.7)	111/171 (64.9)	2156/3583 (60.2)	391/951 (41.1)	77/136 (56.6)	205/404 (50.7)
Other/Not Reported	133/226 (58.8)	101/138 (73.2)	42/69 (60.9)	276/433 (63.7)	10/18 (55.6)	12/16 (75.0)	NA
Ethnicity							
Hispanic or Latino	345/642 (53.7)	99/135 (73.3)	44/71 (62.0)	488/848 (57.5)	44/109 (40.4)	24/40 (60.0)	NA
Not Hispanic or Latino	1932/3309 (58.4)	NA	12/22 (54.5)	1944/3331 (58.4)	337/893 (37.7)	99/173 (57.2)	24/57 (42.1)
Others (Not Reported)	241/430 (56.0)	136/215 (63.3)	153/286 (53.5)	530/931 (56.9)	81/181 (44.8)	NA	185/352 (52.6)

Source: Adapted from Tables 3.6.1. 3.6.2, 3.7.1, 3.7.2, 3.8.1, 3.8.2, 3.9.1 and 3.9.2 (Appendix 1), Response to Comments RFI10, Module 1.11.3, STN125678/0.14.

Note: AD=atopic dermatitis; N=number of subjects in the specified group; n=number subjects with the specified AE; m=number of subjects in the specified subgroup; %=n/m X100. NA=not applicable

8.4.7 Local Reactogenicity

8.4.7.1 Overview of Solicited Injection-Site Adverse Reactions

An overview of the pooled solicited injection-site reactions among the Main ISS population is provided in Table 73.

The proportion of MVA-BN vaccinated subjects with any solicited injection-site reactions among all vaccinia naïve subjects (86.8%) was numerically lower compared with vaccinia experienced subjects (93.4%) (Table 73).

AD subjects experienced more grade 3 solicited injection-site reactions (15.4%) compared with other MVA-BN recipients (11.5% for vaccinia naïve healthy, 8.4% for HIV-infected and 4.2% for vaccinia experienced healthy subjects).

The most common solicited injection-site reactions were pain, erythema, swelling and induration among MVA-BN recipients (Table 73).

Table 73: Overview of Pooled Solicited Injection-Site Adverse Reactions – Main ISS (Safety Population)

Category	**MVA-BN** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Naïve AD Subjects	**MVA-BN** Vaccinia Naïve HIV Subjects	**MVA-BN** Total Vaccinia Naïve Subjects	**Placebo** Vaccinia Naïve Healthy Subjects	**ACAM2000** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Experienced Healthy Subjects
	(N=4381) n (%)	(N=350) n (%)	(N=379) n (%)	(N=5110) n (%)	(N=1183) n (%)	(N=213) n (%)	(N=409) n (%)
Any Injection-Site Reaction	**3841 (87.7)**	**300 (85.7)**	**293 (77.3)**	**4434 (86.8)**	**411 (34.7)**	**196 (92.0)**	**382 (93.4)**
≥Grade 3 Reaction	490 (11.2)	54 (15.4)	32 (8.4)	576 (11.3)	12 (1.0)	49 (23.0)	17 (4.2)
Any Pain	3614 (82.5)	283 (80.9)	264 (69.7)	4161 (81.4)	224 (18.9)	135 (63.4)	325 (79.5)
≥Grade 3 Pain	283 (6.5)	53 (15.1)	30 (7.9)	366 (7.2)	10 (0.8)	33 (15.5)	8 (2.0)
Any Erythema	2695 (61.5)	208 (59.4)	168 (44.3)	3071 (60.1)	212 (17.9)	191 (89.7)	331 (80.9)
≥Grade 3 Erythema	182 (4.2)	3 (0.9)	1 (0.3)	186 (3.6)	0 (0.0)	5 (2.3)	8 (2.0)
Any Swelling	2101 (48.0)	179 (51.1)	149 (39.3)	2429 (47.5)	65 (5.5)	138 (64.8)	275 (67.2)
≥Grade 3 Swelling	38 (0.9)	1 (0.3)	1 (0.3)	40 (0.8)	0 (0.0)	1 (0.5)	3 (0.7)
Any Induration	1919 (43.8)	2 (0.6)	9 (2.4)	1930 (37.8)	50 (4.2)	132 (62.0)	288 (70.4)
≥Grade 3 Induration	90 (2.1)	0 (0.0)	0 (0.0)	90 (1.8)	0 (0.0)	0 (0.0)	2 (0.5)
Any Pruritus	1688 (38.5)	71 (20.3)	70 (18.5)	1829 (35.8)	119 (10.1)	179 (84.0)	131 (32.0)
≥Grade 3 Pruritus	56 (1.3)	0 (0.0)	2 (0.5)	58 (1.1)	2 (0.2)	18 (8.5)	3 (0.7)

Source: Adapted from Tables 10 (page 38-39) and 11 (page 41), Integrated Summary of Safety, Module 5.3.5.3, and Tables 3.3.1 and 3.3.2 (Appendix 1), STN125678/0.7.
Note: AD=atopic dermatitis; N=number of subjects in the specified group; n=number subjects with the specified AE; %=n/N X100.

8.4.7.2 Subgroup Analyses of Solicited Injection-Site Reactions

Subpopulation analyses of solicited injection-site reactions based on the safety population of pooled Main ISS are presented in Table 74.

Among the vaccinia naïve subjects vaccinated with MVA-BN, percentages of subjects with solicited injection-site reactions were similar between different ethnicity groups (ranging from 83% to 88%). More female subjects (92.1%) reported solicited injection-site reactions compared with male subjects (81.7%), and more subjects 18 to 40 years of age (87%) experienced injection-site reactions compared with subjects >40 years of age (78%). Percentages of subjects with solicited injection-site reactions among Asian (76.5%) and black/African American (76.9%) subjects were numerically lower compared with white (89.6%), American Indian/Alaska Native (87.5%), other/not reported (87.1%) and Native Hawaiian or Other Pacific Islander (85.7%) subjects.

Among vaccinia experienced subjects vaccinated with MVA-BN, percentages of subjects with solicited injection-site reactions were similar between males and females. More subjects 18 to 55 years (95.5%) reported solicited systemic reactions compared with subjects > 55 years of age (80.7%).

Table 74: Subgroup Analyses of Solicited Injection-Site Adverse Reactions – Main ISS (Safety Population)

Subgroup	**MVA-BN** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Naïve AD Subjects	**MVA-BN** Vaccinia Naïve HIV Subjects	**MVA-BN** Total Vaccinia Naïve Subjects	**Placebo** Vaccinia Naïve Healthy Subjects	**ACAM2000** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Experienced Healthy Subjects
	(N=4381) n/m (%)	(N=350) n/m (%)	(N=379) n/m (%)	(N=5110) n/m (%)	(N=1183) n/m (%)	(N=213) n/m (%)	(N=409) n/m (%)
Age							
18-40 years of age	3813/4353 (87.6)	299/349 (85.7)	207/261 (79.3)	4319/4963 (87.0)	410/1178 (34.8)	195.212 (92.0)	NA
>40 years of age	28/28 (100.0)	1/1 (100.0)	86/118 (72.9)	115/147 (78.2)	1/5 (20.0)	1/1 (100.0)	NA
18-55 years of age	NA	NA	NA	NA	NA	NA	336/352 (95.5)
>55 years of age	NA	NA	NA	NA	NA	NA	46/57 (80.7)
Sex							
Male	1807/2180 (82.9)	94/127 (74.0)	237/311 (76.2)	2138/2618 (81.7)	182/537 (33.9)	168/184 (91.3)	158/169 (93.5)
Female	2034/2201 (92.4)	206/223 (92.4)	56/68 (82.4)	2296/2492 (92.1)	229/646 (35.4)	28/29 (96.6)	224/240 (93.3)
Race							
American Indian or Alaska Native	20/23 (87.0)	NA	1/1 (100.0)	21/24 (87.5)	4/7 (57.1)	5/6 (83.3)	NA
Asian	96/113 (85.0)	29/51 (56.9)	2/2 (100.0)	127/166 (76.5)	8/20 (40.0)	12/12 (100.0)	1/3 (33.3)
Black/African American	553/715 (77.3)	32/33 (97.0)	94/135 (69.6)	679/883 (76.9)	39/184 (21.2)	34/40 (85.0)	2/2 (100.0)
Native Hawaiian or Other Pacific Islander	16/19 (84.2)	1/1 (100.0)	1/1 (100.0)	18/21 (85.7)	1/3 (33.3)	1/3 (33.3)	NA
White	2965/3285 (90.3)	111/127 (87.4)	136/171 (79.5)	3212/3583 (89.6)	348/951 (36.6)	128/136 (94.1)	379/404 (93.8)
Other/Not Reported	191/226 (84.5)	127/138 (92.0)	59/69 (85.5)	377/433 (87.1)	11/18 (61.1)	16/16 (100.0)	NA
Ethnicity							
Hispanic or Latino	518/642 (80.7)	126/135 (93.3)	60/71 (84.5)	704/848 (83.0)	36/109 (33.0)	37/40 (92.5)	NA
Not Hispanic or Latino	2919/3309 (88.2)	NA	15/22 (68.2)	2934/3331 (88.1)	308/893 (34.5)	159/173 (91.9)	46/57 (80.7)
Others (Not Reported)	404/430 (94.0)	174/215 (80.9)	218/286 (76.2)	796/931 (85.5)	67/181 (37.0)	NA	336/352 (95.5)

Source: Adapted from Tables 3.6.1. 3.6.2, 3.7.1, 3.7.2, 3.8.1, 3.8.2, 3.9.1 and 3.9.2 (Appendix 1), Response to Comments RFI10, Module 1.11.3, STN125678/0.14.

Note: AD=atopic dermatitis; N=number of subjects in the specified group; n=number subjects with the specified AE; m=number of subjects in the specified subgroup; %=n/m X100. NA=not applicable

8.4.8 Adverse Events of Special Interest

8.4.8.1 Analyses of Adverse Events of Special Interest in Pooled Main ISS Population

Overview of AESIs in the Pooled Main ISS Population

Proportions of subjects with any AESIs by SOC and PT reported in the Main ISS population after MVA-BN vaccination are presented in Table 75.

Overall, 74 subjects out of 5519 (1.3%) MVA-BN recipients regardless of previous smallpox vaccination and healthy status reported 87 AESIs.

Among vaccinia-naïve healthy subjects, stratified by treatment group (placebo, MVA-BN, or ACAM2000), the percentage of MVA-BN recipients with any AESI was slightly higher (0.7%) than those of placebo recipients (0.3%) or ACAM2000 recipients (0.5%) (Table 75).

AESIs stratified by previous smallpox vaccination status (naïve and experienced) and within the vaccinia-naïve population AESIs were further stratified by health status (healthy, AD and HIV). In the vaccinia-experienced analysis group, all subjects were healthy participants. Across the healthy populations, the percentages of subjects with any AESI were 0.7% among healthy vaccinia-naïve subjects and 2.0% in healthy vaccinia-experienced subjects. More AESIs were reported among MVA-BN recipients who had atopic dermatitis (6.3%) or were HIV-positive (3.7%).

The increased rates of reported AESIs among AD or HIV-infected subjects were largely attributable to the higher incidence of post-vaccination elevation of Troponin observed in trials POX-MVA-008 and POX-MVA-011.

Reviewer's comment: *Most of the clinical trials under the drug development program were conducted without a placebo control for the relevant populations, and there was no placebo-controlled study among the vaccinia-experienced population. It is difficult to determine if the numerically higher proportions of subjects with AESIs among AD or HIV-infected subjects can be attributed to MVA-BN vaccination, introduction of a new Troponin assay in these studies or random variations that cannot be classified as such due to lack of appropriate placebo control.*

The most common AESIs in PT were elevated troponin I [27 (0.5%) subjects; all vaccinia-naïve subjects], palpitations [6 subjects in the vaccinia-naïve population (0.1%), and 6 subjects in the vaccinia-experienced population (1.5%)], and tachycardia [8 subjects in the vaccinia-naïve population (0.2%) and 2 in the vaccinia-experienced population (0.5%)]. All other individual AESIs were reported in smalls numbers of subjects (n = 1–3).

The majority of AESIs were mild in intensity; there were 4 subjects with moderate AESIs (2 events of palpitations and 1 each of elevated troponin I and chest pain) and 3 subjects with 4 severe AESIs (1 subject with coronary artery disease and coronary artery stenosis, and 1 subject each with elevated troponin I and chest pain; all in vaccinia-naïve subjects).

Table 75: Subjects with Any Adverse Events of Special Interest by System Organ Class and Preferred Term (Main ISS Safety Population)

System Organ Class Preferred Term	**Placebo** Vaccinia Naïve Healthy Subjects	**ACAM2000** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Naïve AD Subjects	**MVA-BN** Vaccinia Naïve HIV Infected Subjects	**MVA-BN** All Vaccinia Naïve Subjects	**MVA-BN** Vaccinia Experienced Healthy Subjects
	(N=1183) **n (%)**	**(N=213)** **n (%)**	**(N=4381)** **n (%)**	**(N=350)** **n (%)**	**(N=379)** **n (%)**	**(N=5110)** **n (%)**	**(N=409)** **n (%)**
Any	**4 (0.3)**	**1 (0.5)**	**30 (0.7)**	**22 (6.3)**	**14 (3.7)**	**66 (1.3)**	**8 (2.0)**
Cardiac disorders	**4 (0.3)**	**0 (0.0)**	**18 (0.4)**	**3 (0.9)**	**7 (1.6)**	**27 (0.5)**	**8 (2.0)**
Palpitations	2 (0.2)	0(0.0)	4 (0.1)	1 (0.3)	1 (0.3)	6 (0.1)	6 (1.5)
Bundle branch block right	1 (0.1)	0 (0.0)	2 (0.0)	0 (0.0)	0 (0.0)	2 (0.0)	0 (0.0)
Pericardial effusion	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)
Tachycardia	0 (0.0)	0 (0.0)	5(0.1)	1 (0.3)	2 (0.5)	8 (0.2)	2 (0.5)
Sinus Tachycardia	0 (0.0)	0 (0.0)	2 (0.0	0 (0.0)	0 (0.0)	2 (0.0)	0 (0.0)
Wolff-Parkinson-White syndrome	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)	1 (0.3)	2 (0.0)	0 (0.0)
AV block first degree	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0	0 (0.0)	1 (0.0)	0 (0.0)
Bradycardia	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)
Pericarditis	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)
Sinus arrhythmia	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)
Supraventricular extrasystoles	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)
Coronary artery disease	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	1 (0.0)	0 (0.0)
Coronary artery stenosis	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0	1 (0.3)	1 (0.0)	0 (0.0)
Extrasystoles	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	1 (0.0)	0 (0.0)
Ventricular extrasystoles	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)	1 (0.0)	0 (0.0)
General disorders and administration site conditions	**0 (0.0)**	**1 (0.5)**	**3 (0.1)**	**0 (0.0)**	**1 (0.3)**	**4 (0.1)**	**0 (0.0)**
Chest discomfort	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	1 (0.3)	1 (0.0)	0 (0.0)
Chest pain	0 (0.0)	0 (0.0)	3 (0.1)	0 (0.0)	0 (0.0)	3 (0.1)	0 (0.0)
Investigations	**0 (0.0)**	**0 (0.0)**	**10 (0.2)**	**19 (5.4)**	**7 (1.8)**	**36 (0.7)**	**0 (0.0)**
Troponin I increase	0 (0.0)	0 (0.0)	5 (0.1)	17 (4.9)	5 (1.3)	27 (0.5)	0 (0.0)
ECG abnormal	0 (0.0)	0 (0.0)	2 (0.0)	0 (0.0)	0 (0.0)	2 (0.0)	0 (0.0)
ECG ST segment elevation	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)	1 (0.3)	2 (0.0)	0 (0.0)
ECG ST segment abnormal	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)
ECG T wave abnormal	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)
Cardiac murmur	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	1 (0.0)	0 (0.0)
ECG QT prolonged	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)	1 (0.0)	0 (0.0)
ECG T wave inversion	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)	1(0.0)	0 (0.0)

Source: Adapted from Tables 4.7.8.1 and 4.7.8.2, Response to IR 10, Module 1.11.3, STN125678/0.14

Note: AD=atopic dermatitis; ECG=electrocardiogram; AV=atrioventricular; N=number of subjects in specific group; n=number of subjects with specific event.

Subgroup Analyses of AESIs in Main ISS Population

Subgroup analyses of AESIs stratified by age, sex, and race as well as by treatment and healthy status are presented in Tables 76.

Among healthy, vaccinia-naïve subjects, the proportion of subjects with AESIs was slightly higher in the older age group (> 40 years, 10.7%) compared to the subjects in the younger age group (18–40 years, 0.6%), while more subjects with AESIs were reported in the younger age groups among healthy, vaccinia-experienced subjects (2.3% among subjects 18-55 years of age vs. 0% among subjects > 55 years of age) and vaccinia-naive subjects with AD (6.3% among subjects 18-40 years of age vs. 0% among subjects > 40 years of age) (Tables 76). Since the older age subgroups were quite small compared to the younger age subgroup, these observed differences may be random variations.

Males tended to report more AESIs compared to females among all MVA-BN recipients.

Among vaccinia-naïve AD subjects, the proportion of subjects with AESIs was slightly higher in the African American race group (18.2%) compared with the other race groups (ranging from 3.1% to 9.8%) (Table 76). No other stand out differences were observed when the data were stratified by race and ethnicity.

Reviewer's comment: *Since the number of AESIs and sizes of subgroups were small, the differences among the subgroups may not be clinically meaningful and should be interpreted with caution.*

Table 76: Subgroup Analyses of Adverse Events of Special Interest – Main ISS Population

Subgroup	**Placebo** Vaccinia Naïve Healthy Subjects	**ACAM2000** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Naïve AD Subjects	**MVA-BN** Vaccinia Naïve HIV Subjects	**MVA-BN** Total Vaccinia Naïve Subjects	**MVA-BN** Vaccinia Experienced Healthy Subjects
	(N=1183) n/m (%)	(N=213) n/m (%)	(N=4381) n/m (%)	(N=350) n/m (%)	(N=379) n/m (%)	(N=5110) n/m (%)	(N=409) n/m (%)
Age							
18-40 years of age	4/1178 (0.3)	1/212 (0.5)	27/4353 (0.6)	22/349 (6.3)	10/261 (3.8)	59/4963 (1.2)	NA
>40 years of age	0/5 (0.0)	0/1 (0.0)	3/28 (10.7)	0/1 (0.0)	4/118 (3.4)	7/147 (4.8)	NA
18-55 years of age	NA	NA	NA	NA	NA	NA	8/352 (2.3)
>55 years of age	NA	NA	NA	NA	NA	NA	0/57 (0.0)
Sex							
Male	1/537 (0.2)	0/184 (0.0)	19/2180 (0.9)	9/127 (7.1)	12/311 (3.9)	40/2618 (1.5)	4/169 (2.4)
Female	3/646 (0.5)	1/29 (3.4)	11/2201 (0.5)	13/223 (5.8)	2/68 (2.9)	26/2492 (1.0)	4/240 (1.7)
Race							
Asian	1/20 (5.0)	0/12 (0.0)	0/113 (0.0)	5/51 (9.8)	0/2 (0.0)	5/166 (3.0)	0/3 (0.0)
Black/African American	0/184 (0.0)	0/40 (0.0)	3/715 (0.4)	6/33 (18.2)	3/135 (2.2)	12/883 (1.4)	0/2 (0.0)
White	3/951 (0.3)	1/136 (0.7)	23/3285 (0.7)	4/127 (3.1)	6/171 (3.5)	33/3583 (0.9)	8/404 (2.0)
Other/Not Reported	NA	NA	4/223 (1.8)	7/138 (5.1)	5/69 (7.2)	16/430 (3.7)	NA
Ethnicity							
Hispanic or Latino	0/109 (0.0)	0/40 (0.0)	5/642 (0.8)	7/135 (5.2)	5/71 (7.0)	17/848 (2.0)	NA
Not Hispanic or Latino	1/893 (0.1)	1/173 (0.6)	15/3309 (0.5)	NA	0/22(0.0)	15/3331 (0.5)	0/57 (0.0)
Others (Not Reported)	3/181 (1.7)	NA	10/430 (2.3)	15/215 (7.0)	9/286 (3.1)	34/931 (3.7)	8/352 (2.3)

Source: Adapted from Tables 4.7.9.1, 4.7.9.2, 4.7.9.3, 4.7.10.1, 4.7.10.2, 4.7.11.1, 4.7.11.2, 4.7.12.1, and 4.7.12.2 (Appendix 1), Response to Comments RFI10, Module 1.11.3, STN125678/0.14, and Response to IR24 Module 1.11.3, STN125678/0/35.

Note: AD=atopic dermatitis; N=number of subjects in the specified group; n=number subjects with the specified AE; m=number of subjects in the specified subgroup; %=n/m X100. NA=not applicable

8.4.8.2 Analyses of Adverse Events of Special Interest in the Pooled ISS Population

Overview of AESIs in the Pooled ISS Population

AESIs stratified by treatment group, previous smallpox vaccination status and health status reported from all 22 clinical trials following vaccination are presented in Table 77.

Four subjects out of 1206 placebo recipients (0.3%) experienced AESIs, and one subject out of 213 ACAM2000 recipients (0.5%) experienced an AESI. Across all MVA-BN vaccinated subjects including vaccinia-naïve and vaccinia-experienced subjects, 111 subjects out of 7859 subjects (1.4%) experienced at least one AESIs. For all vaccinia-naïve subjects, 95 out of 7093 MVA-BN recipients (1.3%) experienced AESIs; for all vaccinia-experienced subjects, 16 out of 766 MVA-BN recipients (2.1%) experienced AESIs.

Among the vaccinia-naïve population, AD subjects and HIV-infected subjects reported more AESIs (5.8% for AD subjects and 3.6% for HIV-infected subjects) compared with healthy subjects (0.9%). The more frequent AESIs observed in AD subjects and HIV-infected subjects were largely attributed to the increased number of subjects with post-vaccination elevation of troponin (refer to Troponin Analysis below).

Among the vaccinia-experienced population, the percentage of subjects experienced AESIs was slightly higher among HIV-injected subjects (2.4%) compared with healthy subjects (1.4%).

Overall, these findings in AESIs based on the ISS of all 22 clinical trials are in line with the analysis for the Main ISS of pooled 12 clinical trials as presented above.

Table 77: Subjects with Any Adverse Events of Special Interest by System Organ Class and Preferred Term (ISS Safety Population)

System Organ Class Preferred Term	**Placebo** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Naïve AD Subjects	**MVA-BN** Vaccinia Naïve HIV Infected Subjects	**MVA-BN** All Vaccinia Naïve Subjects	**MVA-BN** Vaccinia Experienced Healthy Subjects	**MVA-BN** Vaccinia Experienced HIV infected Subjects	**MVA-BN** Vaccinia Experienced Subjects
	(N=1206) n (%)	**(N=6216) n (%)**	**(N=381) n (%)**	**(N=478) n (%)**	**(N=7093)[a] n (%)**	**(N=532) n (%)**	**(N=218) n (%)**	**(N=766)[b] n (%)**
Any	**4 (0.3)**	**55 (0.9)**	**22 (5.8)**	**17 (3.6)**	**95 (1.3)**	**13 (2.4)**	**3 (1.4)**	**16 (2.1)**
Cardiac disorders	**4 (0.3)**	**28 (0.5)**	**3 (0.8)**	**7 (1.5)**	**38 (0.5)**	**12 (2.3)**	**2 (0.9)**	**14 (1.8)**
Palpitations	2 (0.2)	8 (0.1)	1 (0.3)	1 (0.2)	10 (0.1)	6 (1.1)	0 (0.0)	6 (0.8)
Bundle branch block right	1 (0.1)	2 (0.0)	0 (0.0)	0 (0.0)	2 (0.0)	1 (0.2)	0 (0.0)	1 (0.1)
Pericardial effusion	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)	0 (0.0)	1 (0.1)
Tachycardia	0 (0.0)	8 (0.1)	1 (0.3)	2 (0.4)	11 (0.2)	2 (0.4)	1 (0.5)	3 (0.4)
Sinus Tachycardia	0 (0.0)	3 (0.0	0 (0.0)	0 (0.0)	3 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Wolff-Parkinson-White syndrome	0 (0.0)	1 (0.0)	0 (0.0)	1 (0.2)	2 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
AV block first degree	0 (0.0)	1 (0.0)	0 (0.0	0 (0.0)	1 (0.0)	1 (0.2)	0 (0.0)	1 (0.1)
AV block second degree	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Acute myocardial infarction	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Bradycardia	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pericarditis	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Sinus arrhythmia	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Coronary artery disease	0 (0.0)	0 (0.0)	0 (0.0	1 (0.3)	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Coronary artery stenosis	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cardiac failure congestive	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Extrasystoles	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Ventricular extrasystoles	0 (0.0)	0 (0.0)	1 (0.3)	1 (0.2)	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Supraventricular extrasystoles	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)	1 (0.2)	0 (0.0)	1 (0.1)
Bundle branch block	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	1 (0.1)
Bundle branch block left	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)	0 (0.0)	1 (0.1)
General disorders and administration site conditions	**0 (0.0)**	**12 (0.2)**	**0 (0.0)**	**1 (0.2)**	**13 (0.2)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**
Chest discomfort	0 (0.0)	2 (0.0)	0 (0.0)	1 (0.3)	3 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Chest pain	0 (0.0)	10 (0.2)	0 (0.0)	0 (0.0)	10 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Investigations	**0 (0.0)**	**19 (0.3)**	**19 (5.0)**	**10 (2.1)**	**49 (0.7)**	**1 (0.2)**	**1 (0.5)**	**2 (0.3)**
Troponin I increase	0 (0.0)	6 (0.1)	17 (4.5)	8 (1.7)	31 (0.4)	0 (0.0)	1 (0.5)	1 (0.1)
ECG change	0 (0.0)	7 (0.1)	0 (0.0)	0 (0.0)	8 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
ECG abnormal	0 (0.0)	3 (0.0)	0 (0.0)	0 (0.0)	3 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
ECG ST segment elevation	0 (0.0)	1 (0.0)	0 (0.0)	1 (0.2)	2 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
ECG ST segment abnormal	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
ECG T wave abnormal	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cardiac murmur	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	1 (0.0)	1 (0.2)	0 (0.0)	1 (0.1)
ECG QT prolonged	0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
ECG T wave inversion	0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)	1(0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Source: Adapted from Tables 5.4.1.1 and 5.4.1.2, Response to IR 10, Module 1.11.3, STN125678/0.18, and Response to IR24 Module 1.11.3, STN125678/0/35.
Note: AD=atopic dermatitis; ECG=electrocardiogram; AV=atrioventricular; N=number of subjects in specific group; n=number of subjects with specific event.
[a] Including 14 subjects with allergic rhinitis and 4 subjects with hematopoietic stem cell transplants (HSCT), [b] Including 16 HSCT subjects.

Troponin Analysis

As described above, AESI in terms of troponin elevation was defined as a troponin value >2 X ULN. The ISS also includes integrated analyses of all abnormal troponins (i.e., troponin >ULN) following MVA-BN vaccination from all clinical studies where post-vaccination troponin laboratory data were collected.

The availability of post-vaccination troponin values varies across studies. Six of the 22 studies (POX-MVA-001, -03X, -004, -009, -029 and -036) did not collect any post-vaccination troponin as part of the laboratory data, thus these studies were excluded from the pooled ISS of troponin elevation. As a result, the analysis included 5698 vaccinia-naïve subjects who received any dosing regimen or formulation of MVA-BN and had post-vaccination troponin data. A total of 1201 vaccinia-naïve placebo recipients and 213 vaccinia-naïve ACAM2000 recipients were also included in this analysis.

As seen from Table 78, overall 149 out of 5746 subjects (2.6%) who received MVA-BN experienced post-vaccination elevation of troponin. As comparison, 1 subject who received ACAM2000 (0.47%) and no placebo recipient experienced post-vaccination elevation of troponin.

Table 78: Summary of Vaccinia-Naïve Subjects with Post-Vaccination Elevation of Troponin (ISS Population)

Troponin Test	MVA-BN Healthy	MVA-BN AD	MVA-BN HIV	All MVA-BN Recipients	Placebo Healthy	ACAM2000 Healthy
"Conventional" Test[a] (N)	4561	98	135	4812	1201	213
Troponin > ULN n (%)	3 (0.07)	0 (0.0)	2 (1.48)	5 (0.10)	0 (0.0)	1 (0.47)
Troponin >2X ULN n (%)	2 (0.04)	0 (0.0)	1 (0.74)	3 (0.06)	0 (0.0)	0 (0.0)
"High Sensitivity" Test[a] (N)	285	300	349	934	NA	NA
Troponin > ULN n (%)	50 (17.54)	54 (18.0)	40 (11.46)	144 (15.42)	NA	NA
Troponin >2X ULN n (%)	3 (1.05)	17 (5.67)	7 (2.01)	27 (2.89)	NA	NA

Source: Adapted from Table 6 (page 34), Module 5.3.5.3_ISS Summary Description Data Tables (Response to Comments 0008 ISS), STN125678/0.7

[a]A total of 48 subjects from studies POX-MVA-008 (40 subjects) and POX-MVA-011 (8 subjects) had both post-vaccination "conventional" and "high sensitivity" troponin tests. These 48 subjects were included in both incidence rate calculation of the respective test.

Among the 149 MVA-BN recipients with post-vaccination elevation of troponin, 144 subjects were from two studies, POX-MVA-008 and -011. For all these 144 subjects, their troponin was assessed with a new "high sensitivity" troponin test. The "high sensitivity" troponin test was introduced during the two trials. The majority of the study subjects were assessed using the "high sensitivity" test, and

a fraction of subjects were assessed for troponin using both regular test and high sensitivity test (see footnote of Table 78).

Among the 188 subjects whose troponin was assessed using "conventional" troponin assays [161 subjects (94 healthy and 67 AD subjects) in POX-MVA-008 and 27 subjects (19 healthy and 8 HIV-infected subjects) in POX-MVA-011], no subject showed post-vaccination elevation of troponin.

All the 144 subjects with abnormal post-vaccination troponin were assessed using the "high sensitivity" troponin test. Table 79 presents the percentages of subjects with post-vaccination elevation of troponin determined by the "high sensitivity" test. The percentages of subjects with abnormal post-vaccination troponins were similar between AD subjects or HIV-infected subjects and healthy subjects enrolled in these studies, while the proportion of subjects with post-vaccination of troponin > 2 X ULN was higher among AD subjects and HIV-infected subjects.

Table 79: Percentage of Subjects with Post-Vaccination Elevation of Troponin Determined by High Sensitivity Troponin Test in Studies POX-MVA-008 and -011 (Safety Population)

	Troponin > ULN n/N (%)	**Troponin > 2 X ULN n/N (%)**
POX-MVA-008		
All Subjects	94/512 (18.4)	19/512 (3.7)
Healthy Subjects	40/212 (18.9)	2/212 (0.9)
AD Subjects	54/300 (18.0)	17/300 (5.7)
POX-MVA-011		
All Subjects	50/422 (11.8)	8/422 (1.9)
Healthy Subjects	10/73 (13.7)	1/73 (1.4)
HIV-Infected Subjects	40/349 (11.5)	7/349 (2.0)

Source: Adapted from Tables 7.1.2 and 7.2.2, Module 5.3.5.3_ISS Section 7, STN125678/0.
[a]A total of 48 subjects from studies POX-MVA-008 (40 subjects) and POX-MVA-011 (8 subjects) had both post-vaccination regular and high sensitivity troponin tests. These 48 subjects were included in both incidence rate calculation of the respective test.

Reviewer's Comment: *Section 5.2 Troponin I (ISS Summary Description Data Tables, Module 5.3.5.3, STN125678/0.7) states that 6 (POX-MVA-001, -03X, -004, -009, -029 and -036) of the 22 studies did not collect any post-vaccination troponin data and thus these 6 studies were excluded from the pooled ISS of troponin. As a result, the analysis included 5698 vaccinia-naïve subjects, and no vaccinia-experienced subjects were included in the pooled ISS of troponin because myopericarditis is not a known risk factor in vaccinia-experienced individuals. However, five other studies (POX-MVA-005, -010, -011, -023 and -024) enrolled vaccinia-experienced subjects and post-vaccination troponin data were collected. An IR (#26) was sent on 30 May 2019 to the applicant for clarification.*

The applicant submitted its response to STN125678/0.39. The results are presented in Table 80. Among 732 vaccinia-experienced subjects vaccinated with MVA-BN, 16 subjects experienced post-vaccination elevation of troponin (2.2%). Among the 16 subjects with post-vaccination elevation of troponin, 15 subjects were assessed with High Sensitivity Troponin Assay and all these 15 subjects were HIV infected subjects from POX-MVA-011. The results are similar to those in vaccinia-naïve population.

Table 80: Summary of Vaccinia-Experienced Subjects with Troponin Elevation Post MVA-BN Vaccination (ISS Population)

Troponin Test	Healthy Subjects	HIV-Infected Subjects
"Conventional" Test (N)	505	96
Troponin > ULN, n (%)	0 (0.0)	1 (1.0)
"High Sensitivity" Test (N)	7	124
Troponin > ULN, n (%)	0 (0.0)	15 (6.5)
Troponin >2X ULN, n (%)	0 (0.0)	4 (0.8)

Source: Adapted from Tables 7.1.5 and 7.2.5, Module 1.11.3-Response to IR #26, STN125678/0.39

It is generally understood that myopericarditis is not a known risk factor in vaccinia-experienced individuals. However, Study POX-MVA-008 showed that subjects following the second dose of MVA-BN more likely experienced post vaccination troponin elevation than after the first dose vaccination (Section 9.2). An integrated analysis of post vaccination troponin elevation stratified by treatment period as well as types of troponin assays, and study population was conducted. The results are presented in Table 81.

Table 81: Rates of Subjects with Abnormal Post-Vaccination Troponin Stratified by Post Dose 1 and Post Dose 2 of MVA-BN

Population and Troponin Assays	Subjects with Troponin >ULN Post Dose 1 n/N (%)	Subjects with Troponin >ULN Post Dose 2 n/N (%)
All Vaccinia-Naïve Population		
All Troponin Assays	40/5563 (0.7%)	77/1603 (4.8%)
"Conventional" Troponin Assays	3/4697 (0.1%)	0/737 (0%)
"High Sensitivity" Troponin Assays	37/866 (4.3%)	77/872 (8.8%)
Healthy, Vaccinia-Naïve Population		
All Troponin Assays	12/4707 (0.3%)	27/785 (3.4%)
"Conventional" Troponin Assays	3/4456 (0.1%)	0/518 (0%)
"High Sensitivity" Troponin Assays	10/251 (4.0%)	27/271 (10.0%)
HIV-infected, Vaccinia-Naïve Population		
All Troponin Assays	15/467 (3.2%)	15/442 (3.4%)
"Conventional" Troponin Assays	0/128 (0%)	0/123 (0%)
"High Sensitivity" Troponin Assays	15/339 (4.4%)	15/319 (4.7%)
AD, Vaccinia-Naïve Population		
All Troponin Assays	13/373 (3.5%)	35/361 (9.7%)
"Conventional" Troponin Assays	0/97 (0%)	0/81 (0%)
"High Sensitivity" Troponin Assays	13/276 (4.7%)	35/282 (12.4%)
Healthy, Vaccinia-Experienced Population*	0/510 (0%)	0/63 (0%)
HIV-Infected, Vaccinia-Experienced Population*	2/208 (1.0%)	6/140 (4.3%)

Source: Adapted from Tables 13.1.1.1-3, 13.1.2.1-3, 13.1.3.1-3, and 13.1.4.1-3, STN125678/0.13, Module 1.11.3_Clinical Information Amendment _Response to IR 8.
*Troponin assay was not specified.
ULN=upper limited normal, n=number of subjects with post vaccination troponin elevation, N=number of subjects in the specific group.

As seen from Table 81, among all study populations except for HIV-infected, vaccinia-naïve population, frequencies of subjects with post vaccination troponin elevation were higher after dose 2 of MVA-BN than after dose 1 when all troponin assays were included. Higher rates of subjects with increased troponin post dose 2 of MVA-BN was driven by subjects whose troponin was assessed with "high sensitivity" troponin assays as well as a single study POX-MVA-008. Among the 271 healthy, vaccinia-naïve individuals whose troponin was assessed with "high sensitivity" troponin assays, approximately 200 subjects were from POX-MVA-008 and the remaining subjects from study POX-MVA-011. Given that all the subjects with post vaccination troponin elevation were asymptomatic and the "high sensitivity" troponin assay was not clearly by FDA, the clinical significance of the observed higher rate of post vaccination troponin elevation after dose 2 of MVA-BN is unknown.

Reviewer's comment: *Both study POX-MVA-008 and -011 were completed before 2010. The first high sensitivity troponin assay was cleared by FDA in June 2018 (k172783, Beckman Access hsTnI). We sent several IRs to the applicant for detailed information regarding the "high sensitivity" troponin assays used in these studies, and the applicant was not able to provide the request*

information. It is unknown whether the troponin assays were adequately validated, and whether the observed higher rate of abnormal troponin-I after dose 2 of MVA-BN in study POX-MVA-008 was due to MVA-BN or a random effect.

Treatment Related AESIs (ISS)

Among the 22 studies, 81 subjects reported AESIs that were considered at least possibly related to MVA-BN treatment. Of the 81 subjects, 1 subject each reported pericarditis (Subject (b) (6) , POX-MVA-013), myocardial infarction (Subject (b) (6) , POX-MVA-036), and right bundle branch block (BBB) (Subject (b) (6) , POX-MVA-013), and left BBB (Subject (b) (6) , POX-MVA-011), and 3 subjects experienced palpitation, 4 subjects tachycardia, 5 subjects abnormal ECG changes and 65 subjects troponin elevation (48 subjects in POX-MVA-008 and 17 subjects in POX-MVA-011).

All the AESIs were mild or moderate in intensity except for the pericarditis and the myocardial infarction, and all the AESIs recovered.

Summary of Pericarditis: Subject (b) (6)

Subject (b) (6) was a 32-year-old female subject who received the first injection of MVA-BN on (b) (6) . On (b) (6) , 23 days after administration of MVA-BN, she reported mild chest pain which had continued to minimally worsen in frequency and intensity and started 2 days after an atypically prolonged course of induration (> 20 days) at the initial vaccination site. The subject reported intermittent chest pain discomfort rated as a “5 out of 10” with occasional “7 out of 10” spikes on the left upper chest area, which was significantly improved with an upright posture and worsened by lying down or leaning forward. Additionally, the pain was described as worsening with active inspiration and less intense with expiration. ECG and Troponin were normal on (b) (6) . Physical exam was unremarkable.

The subject was seen by a cardiologist on (b) (6) , with an overall assessment of “Pleuritic and likely due to pericarditis, which could be a complication of the recent smallpox vaccine, with a grossly normal ECHO, with no evidence of pericardial effusion”. A repeat ECG and troponin were normal.

Upon request of the DSMB and the applicant, a viral, bacterial and antinuclear antibody lab (ANA) panel in order to explore alternative etiology was requested. The results from (b) (6) showed no hints for an acute infection, rheumatoid factors were negative; however, titers for Coxsackie B virus were positive.

The event was considered resolved without sequela on (b) (6) , and the subject was discontinued from further vaccination.

Due to the temporal association of the event to the treatment, the investigator reported the event as possibly related to MVA-BN, however, the applicant considered the event unlikely related to the treatment because the time course of the event was not consistent with previous experiences with smallpox vaccines (10-15 days after vaccination).

Reviewer's comment: *This reviewer generally agrees that the event was unlikely related to MVA-BN because of the presence of a potential alternative causality (i.e., Coxsackie B virus infection). However, although most reported cases of myo- pericarditis have occurred during 8-14 days after smallpox vaccination, some cases did occur after 15 days following smallpox vaccination[17].*

Summary of Myocardial Infarction: Subject (b) (6)

Subject (b) (6) was a 30 year old male who experienced non-ST elevation myocardial infarction (MI) without epicardial coronary artery disease (CAD) approximately four months after MVA-BN vaccination. The subject had no history of cardiac disease; however, family history was significant for MI (both grandfathers in their 50s with MI) and blood clots (father). He occasionally smoked cigarettes and consumed alcoholic drinks but had no history of substance use.

On (b) (6), the subject received a single 0.5 mL dose of MVA-BN. He had no systemic reactogenicity post-vaccination on Day 1 through Day 7 but had significantly erythema/redness (maximal diameter of 35 mm on Day 4 and still at 20 mm on Day 14). The subject was not eligible for the second dose of the study vaccine per protocol due to his exfoliative skin condition.

On (b) (6), the subject was started on doxycycline 100 mg daily for malaria prophylaxis for his trip to India. On 14 December 2013, the subject developed diarrhea while in India, for which he took two doses of azithromycin. On (b) (6), upon return to the US, the subject visited an urgent care for his ongoing diarrhea and the azithromycin was switched to ciprofloxacin 1000 mg daily. On 20 December 2013, the diarrhea was mostly resolved except for abdominal cramping.

On (b) (6), the subject presented to an emergency department with acute onset of substernal chest pain and mild diaphoresis which lasted for 2 hours. The pain was rated 7/10 and radiated to the left and right of the sternum, without radiation to the back. The subject was found to have an elevated D-dimer of 629 ng/mL [normal range (NR) 0–400] and troponin of 1.27 and 2.6 ng/mL (NR 0.0–0.1). Serum potassium was at 3.4 mEq/L (NR 3.5–5.2). (Per report, the subject had a baseline serum potassium of 4.4 mEq/L on (b) (6)). He was admitted to the hospital for further evaluation and management. A CT pulmonary angiogram showed no pulmonary embolism. Per site, the subject had

a negative chest x-ray, and normal serial ECGs showing no evidence of ischemia (sinus rhythm, no peaked T waves, and no ST segment changes). Cardiac catheterization on (b) (6) was normal, revealing a normal coronary angiogram and left ventriculogram. Per cardiology recommendation, he was started on diltiazem and continued on aspirin. The subject was observed overnight and remained stable with stable vital signs and with no arrhythmia detected on telemetry. On (b) (6) , the subject was discharged from the hospital with a diagnosis of non-ST elevation MI with normal coronaries and normal ejection fraction. Per the discharge summary, the etiology was unclear. Takotsubo (cardiomyopathy) versus coronary spasm were considered likely etiologies.

The Investigator has assessed the event, non-ST elevation MI without epicardial CAD, as serious and related to the study vaccine.

The applicant considered the event unlikely related to MVA-BN because of the long lead time between the vaccination and event onset as well as the presence of confounding factors such as diarrhea and ciprofloxacin.

Reviewer's comment: *This reviewer agrees with the applicant's assessment that the event was unlikely related to MVA-BN although causality with the vaccination could not be completely ruled out. As described above, the subject had risk factors for ischemic cardiac disorders. The package insert of ciprofloxacin shows association of the antibiotic with angina pectoris and MI. In addition, medical literature also suggests an association between bacterial gastroenteritis with acute onset of myocarditis following campylobacter infection[18, 19] and salmonella infection[20]. All these could be alternative etiologies of the event.*

Individual narratives of AESIs and causality assessment were provided in Module 5.3.5.3 (ISS Section 8, Narrative Summaries), STN125678/0.

Reviewer's comment: *This reviewer has reviewed all the individual narratives of AESIs and concurs with the applicant's assessments for the AESIs not discussed above.*

<u>Subgroup Analyses of AESIs in the ISS Population</u>

AESIs stratified by age, sex, race and ethnicity are presented in Tables 82.

For vaccinia-naïve healthy and vaccinia-experienced healthy subjects, the proportion of subjects with AESIs was higher in the older age group (> 40 years for vaccinia-naive and >55 years for vaccinia-experienced) compared to the subjects in the younger age group (18–40 years or 18-55 years), whereas the opposite was true for vaccinia-naïve AD subjects or HIV-infected subjects (Table 82).

No obvious difference was observed between male and female subjects.

For vaccinia-naïve AD or HIV infected subjects, the proportion of subjects with AESIs was slightly higher in African American race group compared with the other race groups (Table 82). No other significant differences were observed when the data were stratified by race or ethnicity (Table 82). Also, when displayed by treatment group, there were no relevant differences in the stratifications for race and ethnicity between the analyzed groups, neither for overall incidence nor with regard to the pattern of reported AESIs (Table 82).

Reviewer's comment: *Due to the limited numbers of AESIs in each subgroup as well as small sample sizes for some subgroups, the observed differences among subgroups may be random variations. The results from these subgroup analyses need to be interpreted with caution.*

Table 82: Subgroup Analyses of Adverse Events of Special Interest – ISS Population (All Studies)

Subgroup	**Placebo** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Naïve Healthy Subjects	**MVA-BN** Vaccinia Naïve AD Subjects	**MVA-BN** Vaccinia Naïve HIV Subjects	**MVA-BN** Total Vaccinia Naïve Subjects	**MVA-BN** Vaccinia Experienced Healthy Subjects	**MVA-BN** Vaccinia Experienced HIV Infected Subjects	**MVA-BN** Vaccinia Experienced Subjects
	(N=1206) n/m (%)	(N=6216) n/m (%)	(N=381) n/m (%)	(N=478) n/m (%)	(N=7093)[a] n/m (%)	(N=532) n/m (%)	(N=218) n/m (%)	(N=766)[b] n/m (%)
Age								
18-40 years of age	3/1201 (0.2)	52/6213 (0.8)	22/380 (5.8)	13/341 (3.8)	88/6902 (1.3)	NA	NA	NA
>40 years of age	0/5 (0.0)	3/53 (5.7)	0/1 (0.0)	4/137 (2.9)	7/191 (3.7)	NA	NA	NA
18-55 years of age	NA	NA	NA	NA	NA	8/411 (1.9)	3/218 (1.4)	11/640 (1.7)
>55 years of age	NA	NA	NA	NA	NA	5/121 (4.1)	0/0 (0.0)	5/126 (4.0)
Sex								
Male	2/552 (0.4)	30/3069 (1.0)	9/141 (6.4)	13/393 (3.3)	53/3613 (1.5)	7/230 (3.0)	3/175 (1.7)	10/417 (2.4)
Female	1/654 (0.2)	25/3147 (0.8)	13/240 (5.4)	4/85 (4.7)	42/3480 (1.2)	6/302 (2.0)	0/43 (0.0)	6/349 (1.7)
Race								
American Indian or Alaska Native	0/7 (0.0)	0/24 (0.0)	NA	0/1 (0.0)	0/25 (0.0)	NA	NA	NA
Asian	1/21 (4.8)	0/170 (1.2)	5/53 (9.4)	0/2 (0.0)	7/225 (3.1)	0/5 (0.0)	0/1 (0.0)	0/6 (0.0)
Black/African American	1/186 (0.5)	3/911 (0.3)	6/34 (17.6)	6/180 (3.3)	15/1125 (1.3)	0/14 (0.0)	0/68 (0.0)	0/82 (0.0)
Native Hawaiian or Other Pacific Islander	0/3 (0.0)	0/23 (0.0)	0/1 (0.0)	0/2 (0.0)	0/26 (0.0)	NA	NA	NA
White	1/971 (0.1)	45/4801 (0.9)	4/155 (2.6)	6/222 (2.7)	56/5196 (1.1)	13/511 (2.5)	2/122 (1.6)	15/649 (2.3)
Other/Not Reported	0/18 (0.0)	5/287 (1.8)	7/138 (5.1)	5/71 (7.0)	17/496 (3.5)	0/2 (0.0)	1/27 (3.7)	1/29 (3.6)
Ethnicity								
Hispanic or Latino	0/110 (0.0)	6/799 (0.8)	7/135 (5.2)	5/78 (6.4)	18/1012 (1.8)	0/2 (0.0)	1/27 (3.7)	1/29 (3.4)
Not Hispanic or Latino	2/915 (0.2)	34/4529 (0.8)	NA	1/75(1.3)	35/4608 (0.8)	5/122 (4.1)	NA	5/138 (3.6)
Others (Not Reported)	1/181 (0.6)	15/888 (1.7)	15/246 (6.1)	11/325 (3.4)	42/1473 (2.9)	8/408 (2.0)	2/191 (1.0)	10/599 (1.7)

Source: Adapted from Tables 5.4.2.1, 5.4.2.2, 5.4.3.1, 5.4.3.2, 5.4.4.1, 5.4.4.2, 5.4.5.1 and 5.4.5.2 (Appendix 1), Response to Comments RFI10, Module 1.11.3, STN125678/0.18.

Note: AD=atopic dermatitis; N=number of subjects in the specified group; n=number subjects with the specified AE; m=number of subjects in the specified subgroup; %=n/m X100. NA=not applicable

[a] Including 14 subjects with allergic rhinitis and 4 subjects with hematopoietic stem cell transplants (HSCT), [b] Including 16 HSCT subjects.

Analysis of Adverse Events Leading to Withdrawal

Among the 22 clinical trial, 10 trials (i.e., POX-MVA-001, -002, -004, -023, -028, -029, -30, -37, -03X and HIV-POL-002) did not report any subjects who withdrew from study or further vaccination due to AEs. In all other studies, in total 84 AEs in 57 subjects led to withdrawal from the study or from further vaccination.

Reviewer's comment: *The submission (Section 2.1.4.4 AE Leading to Withdrawal, Module 2.7.4, STN125678/0) stated that 88 AEs reported by 61 subjects led to withdrawal, which included 4 AEs reported by 4 placebo recipients. These 4 placebo recipients were excluded from the analysis of AEs leading to withdrawal below.*

Most AEs leading to withdrawal were considered unrelated or unlikely related to study vaccine [55 AEs (65.5%) in 40 subjects (70.2%)]. There was no particular pattern in terms of PT with regard to the nature of the individual AEs leading to withdrawal. Table 83 summarizes the AEs leading to withdrawal that were considered at least possibly related to MVA-BN.

Table 83: Adverse Events Leading to Withdrawal by Studies

Study ID Subject ID	AE	AE Grade	Outcome
POX-MVA-007			
(b) (6)	Increased hepatic enzyme	1	Recovered
POX-MVA-013			
(b) (6)	Arthralgia	1	Recovered
	Injection-site pain	3	Recovered
	Injection-site erythema	1	Recovered
	Injection-site swelling	1	Recovered
	Injection-site induration	1	Recovered
	Injection-site pruritus	3	Recovered
	Headache	3	Recovered
	Injection-site hematoma	1	Recovered
	Injection-site discoloration	1	Ongoing
	Injection-site induration	1	Recovered
	Bundle branch block right	1	Ongoing
	Acute pericarditis	1	Recovered
	Urticaria	2	Recovered
	Pruritus	1	Recovered
POX-MVA-036			
(b) (6)	Acute worsening right shoulder pain	2	Recovered
	Acute worsening chronic low back pain	2	Recovered
	General body rash	2	Recovered
	Hives	2	Recovered
	Angioedema	1	Recovered
	Upper extremities joint pain	2	Recovered
	Hives	2	Recovered
	Sub stern chest tightness	1	Recovered
	Shortness of breath	1	Recovered
POX-MVA-011			
(b) (6)	Left bundle branch block	1	Recovered
	Injection-site dermatitis	2	Ongoing
	Neutrophil count decreased	1	Recovered

Source: Adapted from Table 11, Appendix to ISS, Section 9, Module 5.3.5.3, STN125678/0
Note: Among the 12 studies with reported AEs leading to withdrawal, 7 studies (POX-MVA-005, -006, -008, -010, -024 and -027, and HIV-NEF-004) did have AEs leading to withdrawal that were considered at least possibly related to MVA-BN.

Reviewer's comment: *This reviewer has reviewed the narratives of individual AEs leading to withdrawal and concurs with the applicant's causality assessments.*

8.6 Safety Conclusions

Safety of MVA-BN was assessed in more than 7800 subjects including AD subjects and HIV-infected subjects who received at least one dose of MVA-BN at 1 x 10^8 $TCID_{50}$ or higher in 22 studies under the drug development program. Across all 22 clinical trials and in all populations including those who are contraindicated for replicating vaccinia-based smallpox vaccines such as HIV-

infected subjects, and those for whom replicating vaccinia based smallpox vaccines are not recommended such as AD subjects, the safety profile of MVA-BN was favorable.

The most commonly reported AEs were in the SOC of General Disorders (myalgia 32.1%, fatigue 30.8%, headache 28.9%, nausea 14.0% and chills 8.7%) and Administration Site Conditions (injection-site pain 81.2%, erythema 64.2%, swelling 48.4%, induration 42.2% and pruritus 37.3%), which is comparable to other licensed vaccines administered via the SC route.

No clinically relevant difference in the safety and reactogenicity of MVA-BN was observed between vaccinia-naïve and vaccinia-experienced populations. Moreover, no severe adverse reactions that are potentially associated with replicating vaccinia-based smallpox vaccines was reported following MVA-BN vaccination in populations with an increased risk such as HIV-infected individuals and AD patients. Although differences in AE incidence among the studies were observed, no clear patterns emerged regarding the number and nature of AEs among the different doses and formulations.

The cardiac safety profile of MVA-BN was also favorable. MVA-BN did not increase the risk for developing myo-/pericarditis, which was seen with Dryvax and ACAM2000 (e.g. the incidence of myo-/pericarditis after vaccination with ACAM2000 was about 1/175, as shown in ACAM2000 package insert). Overall, the number of subjects with AESIs in this study program was relatively low, particularly for AESIs with the presence of cardiac symptoms, except for one case of mild pericarditis that was assessed as possibly related to MVA-BN and isolated mild to moderate increases of troponin levels. Among the 22 studies, all the studies except for studies POX-MVA-008 and POX-MVA-011 had a few subjects with post-vaccination elevation of troponin.

In studies POX-MVA-008 and POX-MVA-011, a significant number of subjects (up to 18.4%) showed post-vaccination elevation of troponin regardless of health status of study subjects (i.e., healthy, HIV-infected or AD subjects). The increased proportion of subjects with post-vaccination elevation of troponin has been thought to be related to the use of a more sensitive troponin assay. Among these two studies, troponin was assessed with regular troponin assay in about a third of subjects, and only 3 HIV-infected subjects reported post-vaccination elevation of troponin among this subset subjects.

All subjects with elevated troponin levels underwent a cardiologist workup and no clinically meaningful cardiac abnormalities were identified among these subjects. Since there was no placebo-control in these two studies, the increased proportion of subjects with abnormal troponin is uninterpretable.

Reviewer's comment: *Myocarditis and pericarditis have been reported to be associated with the first and the second generations of live, replicating smallpox*

vaccines. The mechanism of myopericarditis associated with vaccinia virus is unclear. JYNNEOS is a live, non-replicating vaccinia virus-based vaccine. Although one case of pericarditis was reported in BLA 125678/0, assessed as possibly related to the vaccine due to temporal association, most clinical trials under the development program did not show an increased proportion of subjects with abnormal troponin or ECG compared with placebo controls. However, up to 18.4% of subjects in two of the pivotal clinical trials were reported to have abnormal troponins following vaccination without cardiac diagnoses or symptoms consistent with clinical cardiac events. The applicant will be asked to continue monitoring cardiac related events as part of the post-marketing pharmacovigilance plan.

Across the 22 clinical trials, no trends for unexpected and/or serious adverse reactions due to the investigational product were detected.

In addition, none of the historically reported complications of replicating vaccinia based smallpox vaccines, such as vaccinia rash, eczema vaccinatum, generalized vaccinia, progressive vaccinia, erythema multiforme or post-vaccinal encephalitis have been observed in the clinical development program of MVA-BN.

Two deaths were reported from the 22 clinical trials of the study program. One each was reported from POX-MVA-011 (due to overdose of Xanax and benzodiazepine) and POX-MVA-013 (suicide), respectively. None was related to MVA-BN.

9. Additional Clinical Issues

9.1 Special Populations

9.1.1 Human Reproduction and Pregnancy Data

Twenty-nine pregnancies among MVA-BN vaccinated subjects occurred in 7 of the 22 studies: 14 in POX-MVA-013, 1 each in POX-MVA-002 and POX-MVA-004, 3 in POX-MVA-005, 3 in POX-MVA-009, and 2 in POX-MVA-027; and 5 in POX-MVA-008. Among 29 women who became pregnant during the study and follow-up period, 16 women delivered 16 healthy babies without complications (55.2%), 5 reported elective abortions (17.2%), 4 reported spontaneous abortions (13.8%), and 4 pregnancies were lost to follow-up and thus the outcomes of their pregnancies are unknown (13.8%).

Reviewer's comment: *One of the spontaneous abortions was also reported as fetal death. Please refer to Section 6.3.12.4 for details.*

As a comparison, 10 pregnancies were reported in the placebo group, all from POX-MVA-013 trial. Eight of the 10 women delivered healthy babies (80.0%) and 2 women experienced spontaneous abortions (20.0%).

Reviewer's comment: *Based on the limited number of pregnancies reported in the submission, no apparent adverse impact of the vaccine on pregnancy is observed. Consequently, in contrast to replicating smallpox vaccines which are associated with a risk of fetal vaccinia, MVA-BN will not be contraindicated in pregnancy. Nevertheless, the applicant plans to assess pregnancy outcome in the event of mass vaccination under its proposed pharmacovigilance protocol, POX-MVA-039.*

9.1.3 Pediatric Use and PREA Considerations

The applicant submitted an agreed Final Initial Pediatric Study Plan (iPSP) on June 22, 2016 to IND (b) (4), under which the (b) (4) formulation of MVA-BN is being developed. The iPSP applies to the liquid frozen formulation of MVA-BN as well. On July 22, 2016 the FDA informed the applicant that the Agency agreed with the applicant's plan to request a full waiver of the pediatric assessment for MVA-BN because studies in pediatric populations are impossible or highly impracticable, due to the absence of pediatric populations currently at risk for smallpox.

The applicant also requested a full waiver for the monkeypox indication after the applicant agreed to this indication during the BLA review. The full waiver was granted because studies in pediatric populations are impossible or highly impracticable as pediatric populations at risk for monkeypox are limited to small and widely dispersed communities in the deeply forested regions of central Africa.

The agreed iPSP is included in the BLA in Module 1.9 (Pediatric administrative information).

9.1.4 Immunocompromised Patients

Summary of Clinical Review of MVA-BN Study in HIV-Infected Subjects: POX-MVA-011

The clinical trial was an open label, HIV-uninfected controlled, prospective cohort study to assess the safety and immunogenicity of MVA-BN in both vaccinia-naïve and vaccinia-experienced HIV-infected subjects, ages 18-55, with baseline CD4 cell counts ≥ 200-750, compared to HIV-uninfected controls. Five-hundred eighty-one subjects (581) were enrolled, 83% of whom were HIV-infected. Very few HIV-uninfected, vaccinia-experienced subjects were enrolled (n=9), making comparison of safety and immunogenicity data from HIV-infected, vaccinia-experienced subjects difficult. There was also a notable male predominance in the HIV-infected population compared to HIV-uninfected controls.

More than one-third (34.9%) of vaccinia-naïve HIV-infected subjects were seropositive for vaccinia-specific ELISA antibodies at baseline, compared to only 14% of HIV-uninfected controls. ELISA SCRs (defined as either new positive result or a doubling of GMT from baseline) at the time of peak immune response (two weeks after the second dose of MVA-BN) were similar between groups (98%HIV-uninfected vs 97% HIV-infected), though ELISA GMT titers were consistently lower in the HIV-infected group. More HIV-infected subjects were seropositive for PRNT at baseline, compared to HIV-uninfected controls (17% HIV, 7% HIV-uninfected). Unlike ELISA antibody responses, HIV-infected individuals had a notably lower PRNT SCR at Peak Visit, compared to HIV-uninfected controls (58.1% HIV vs 78.9% HIV-uninfected), which suggests the possibility of a less robust functional antibody vaccine response. Seroconversion and GMTs did not differ significantly between CD4 cell count strata though did tend to be lower in subjects with lower CD4 counts. Mean PRNT GMTs at time of peak immune response (2 weeks after the second MVA-BN dose) for both healthy and HIV-infected individuals were at or below the lower limit of quantitation for the assay (20) 20.8 and 13 respectively, which raises doubt about the interpretation and significance of these data.

Reviewer Comment: *There were high rates of baseline ELISA seropositivity (~35% versus 14%) in HIV-infected subjects who had never received a smallpox vaccine ("vaccinia naïve") compared to non-HIV infected control subjects, which raises concerns about poor specificity and potential cross-reactivity of the assay used in this study*. It is also possible that older subjects (>50 years of age) included as "vaccinia naïve" had actually previously received a smallpox vaccine as children during the eradication program and without a visible scar, personal recollection or documentation of vaccination, they may have been misclassified. As rates of seroconversion, defined as at least a doubling of ELISA titers in those individuals who may have been positive at baseline, were similar between groups, HIV-infected individuals do appear to generate an immune response to MVA-BN; whether the response is protective remains unclear. Issues with both ELISA and PRNT assay validation for the assay versions used in this study ultimately preclude inference about vaccine effectiveness in this population and as such, a specific indication for use in HIV subjects may not be included in this label

For vaccinia experienced subjects, ELISA SCRs were similar to the vaccinia-naïve group but PRNT SCRs were notably higher in HIV-infected subjects (76.9%), approaching those of HIV-uninfected controls, suggesting a robust anamnestic functional antibody response to previous vaccination. However, due to low numbers of healthy subjects and PRNT assay issues, statistical comparison of vaccinia-experienced subjects was not possible.

There was one death in this study, due to suicide via benzodiazepine overdose, which was deemed not to be related to MVA-BN by the investigator, and no pregnancies. Thirty-eight SAES were reported in HIV-infected subjects (17

vaccinia-naïve, 6 vaccinia-experienced) and none in HIV-uninfected controls. Most of these fell under the Infectious or Respiratory SOCs. One of these SAEs, pneumonia which occurred 2 days after the 2nd dose of MVA-BN in a 39-year-old HIV-infected, vaccinia-naïve woman, was considered possibly related to MVA-BN. Of note, 1.0% of HIV infected subjects (n=6) were withdrawn from the study due to AE and 7% (n=35) of HIV infected subjects did not receive a second dose of MVA-BN due to worsening of HIV parameters (drop in CD4 count or rise in HIV viral load) after the first dose.

Reviewer Comment: *This reviewer agrees that the death by benzodiazepine overdose was unrelated to MVA-BN vaccination.*

A slightly higher frequency of AESIs, specifically troponin elevations, were reported in HIV-uninfected, vaccinia-naïve subjects (15%) than in HIV-infected, vaccinia naïve subjects (12%) and were reported more frequently following the second dose of MVA-BN in HIV-uninfected vaccinia-naïve subjects [post dose 1: n=1 (1.2%), post dose 2: n=6 (7.1%)] but at similar frequencies following each dose in HIV-infected vaccinia-naïve subjects [post dose 1: n=15 (4.3%), post dose 2: n=15 (4.7%)]. Cardiac AESIs were reported in 6.9% of HIV-infected vaccine experienced subjects with a slightly higher frequency of AESIs after the second dose compared to the first [post dose 1: n=2 (1.6%), post dose 2: n=5 (4.1%)]. No symptomatic cases of myocarditis or pericarditis were reported. Please see additional discussion about troponin elevations and associated assay concerns in Section 8.4.8.2.

Reviewer Comment: *Differences in frequencies of cardiac AESIs between study cohorts (vaccinia-naïve HIV-infected and HIV uninfected) are small and not clinically significant other than to provide reassurance that there is not a higher frequency of cardiac AESIs in HIV-infected individuals.*

Slightly higher rates of reactogenicity, manifested primarily by local injection site reactions, were reported in HIV-uninfected subjects (95%) than those infected with HIV (83%). Both HIV-uninfected and HIV-infected cohorts reported unsolicited AEs in ~67% of subjects, most commonly Administration Site Conditions, abnormal Investigations and Infections and Infestations. Most AEs were mild or moderate, with less than a quarter of subjects reporting a Grade 3 or higher solicited or unsolicited AE, similar between HIV-uninfected and HIV-infected subjects. A higher proportion of HIV-infected subjects (50-60%), regardless of vaccinia status, experienced lab abnormalities during this study compared to HIV-uninfected subjects (20-30%), the majority of which were hematologic abnormalities (decrease in CD4 count/leukopenia, neutropenia and anemia) and previously discussed troponin I elevations. These differences were generally mild, transient and are not unexpected given the underlying immunodeficiency in these subjects.

Overall, the safety profile of MVA-BN in HIV-infected subjects appears to be similar to that of HIV-uninfected individuals, with slightly lower rates of reactogenicity in the HIV-infected population. Lower PRNT GMTs and rates of seroconversion in HIV-infected subjects compared to HIV-uninfected controls suggests that there may be some decreased immunogenicity of MVA-BN in immunocompromised individuals; however, the significance of this is unclear as there is no established antibody threshold for protection against smallpox and PRNT GMTs for a sizeable proportion of vaccinia naïve subjects (both HIV-infected and uninfected) were below the lower limit of quantitation of the assay. Due to these concerns about the validity of the PRNT assay and interpretability of the results, we determined that immunogenicity data from HIV-infected subjects was not suitable for presentation in the package insert and does not support vaccine effectiveness or an indication for use of JYNNEOS specifically in HIV-infected individuals. However, even with the questionable immunogenicity, and by inference, effectiveness, of MVA-BN in this study, the potential benefit of decreased morbidity and mortality associated with potential smallpox infection appears to outweigh the risk, based on safety profile demonstrated in this study, of giving this non-replicating vaccinia vaccine to HIV-infected individuals with CD4 counts above 200 cells/uL, and therefore HIV-infected individuals should not be specifically excluded (contraindicated) from the licensed indication for JYNNEOS.

9.1.5 Geriatric Use

Summary of Clinical Review of MVA-BN Study in Geriatric Population: POX-MVA-024

This study of MVA-BN in 120 smallpox vaccine experienced subjects 56-80 years of age compared the safety and immunogenicity of one versus two doses of study vaccine. Ultimately 102 subjects (50 Group 1, 52 Group 2) received either two doses of MVA-BN or placebo plus one dose of MVA-BN. Forty-two subjects (41%) in the study were over the age of 65, meeting the regulatory definition of geriatric subjects. The primary objective was safety. Immunogenicity endpoints (secondary objective) were proportion of subjects with any immune responses determined by ELISA and PRNT. A response was defined as either the appearance of antibody titers ≥ LLOD for seronegative subjects at baseline or an increase of the antibody titer compared to the baseline titer for subjects with a pre-existing vaccinia specific antibody titer.

Response rates were low, as expected, for Group 2 subjects following placebo vaccination (19%) but responded similarly after MVA-BN (100%). Rates of seroconversion determined by ELISA were calculated post-hoc and were ~83% for both Group 1 and Group 2 at 2 weeks after the second vaccination (Week 6). All subjects over the age of 65 had an increase in antibodies (either ELISA or PRNT) following MVA-BN vaccination with similar SCRs calculated post-hoc.

There were no deaths and 2 SAEs in this study (non-cardiac chest pain and adenocarcinoma of the prostate), neither of which were thought to be related to the study vaccination by the investigator, an assessment with which this reviewer agrees. Five subjects (4 over the age of 65) reported AESIs, primarily ECG abnormalities, without any elevated troponin levels or cases of myopericarditis, despite routine ECG and troponin monitoring at both post-vaccination visits. Most subjects (81-87%) reported at least one adverse event following MVA-BN, with significantly lower rates with placebo vaccination. Injection site reactions were the most commonly reported AE, followed by MSK complaints, Skin conditions and Infections/Infestations. Rates of solicited AEs were lower in the over 65 population (~50%) and there were no unique trends in unsolicited AES in this population.

Given PRNT assay validation issues and the limited clinical significance of ELISA antibody response, immunogenicity data from this study is inadequate to support the use of a single booster dose in elderly vaccine experienced individuals. In summary, MVA-BN appears to be safe in vaccinia-experienced individuals over the age of 55, including the geriatric subpopulation of subjects over 65 years of age, and the overall immunogenicity evidence supports inference of effectiveness, as well as a favorable benefit/risk profile for use of the two-dose regimen in smallpox-vaccine-experienced elderly individuals.

9.2 Aspect of the Clinical Evaluation Not Previously Covered

Summary of Clinical Review of MVA-BN Study in AD Subjects: POX-MVA-008

POX-MVA-008 was an open label, prospective cohort study comparing immunogenicity and safety of two doses of MVA-BN in 350 subjects, age 18-40, diagnosed with mild atopic dermatitis (AD) compared to 282 controls without AD.

The primary endpoint of non-inferiority in subjects with AD in this study was defined as a difference in ELISA seroconversion frequency at 2 weeks after the second dose of MVA-BN between subjects with and without AD with a lower bound of the 97.5% CI better than -5%. Ninety-seven percent (97.3%) of AD subjects (95% CI 94.5-98.9) and 98.5% (95% CI 95.5-99.7) of non-AD subjects met the definition of ELISA seroconversion, for a difference of -1.2%. Non-inferiority criterion was met with a 97.5% LB of -4.3%. Other immunogenicity parameters, including rates of PRNT seroconversion and ELISA and PRNT GMTs appear to be similar between study cohorts, with demonstration of an increase in antibody production above baseline following vaccination in most subjects. However, as average PRNT GMTs for both healthy subjects and subjects with AD were below the level of quantitation of the assay, it is impossible to truly compare the level of functional antibody production between study populations. As there is no established level of protection against smallpox for anti-vaccinia ELISA or PRNT GMTs, effectiveness cannot be inferred from the immunogenicity data specifically for individuals with AD. There are no specific

clinical concerns, however, about diminished vaccine immune response in individuals with atopic dermatitis.

Adverse events were experienced by the majority of subjects during the 29-day period after receiving MVA-BN (95% non-AD control vs 94.6% AD). There were no deaths in this study. About 1% of subjects had an SAE (3 control, 4 AD), all following the 2nd vaccination or during follow-up, and only one of these events was considered at least possibly related to the study vaccine by the sponsor (extraocular muscle paresis 8 days after MVA-BN dose 2; unlikely to be related per this reviewer). No dermatological SAEs were reported. About three-quarters of all subjects reported solicited local AEs (76.7% AD vs 75.8% control) and a higher proportion of subjects with AD reported solicited general AEs (70.1% AD vs 56.4% control), however a low percentage of solicited events, 0.5%, were Grade 3 or higher in severity. Unsolicited AEs were reported by a similar proportion of subjects in each cohort (68.6% AD vs 64.5% control) and the most commonly reported AEs (in >5% subjects) were injection site pruritus (17.0% control vs 28.6% AD), headache (6.7% vs 4.6%) and nasopharyngitis (3.9% vs 6.6%). A higher percentage of unsolicited AEs were considered related to MVA-BN in the AD group (52.4% vs 40% control).

A slightly higher but similar proportion of subjects with AD reported cardiac AESIs (16.6% vs 13.5% control), most of which were elevated troponin-I levels (n= 54, 15.4% AD vs n=37, 13.1% control). A higher number of subjects reported cardiac AESIs after the 2nd MVA-BN dose (n=39 AD [11.9%], n=20 [7.5%] control) compared to the 1st MVA-BN dose (n=21 [6.1%] AD, n= 18 [6.4%] control). Similarly, a higher number of subjects were documented to have elevated troponin-I following the 2nd dose (n= 37 [11.3%] AD, n= 20 [7.5%] control) compared to the 1st dose (n=19 [5.5%] AD, n= 17 [6.0%] control). No symptomatic cases of myocarditis or pericarditis were reported. Please refer to Section 8.4.8.2 for additional discussion about post vaccination troponin elevations and associated assay concerns.

In general, MVA-BN appears to be similarly immunogenic and generally safe, with a slightly increased reactogenicity profile, in patients with a diagnosis of AD as compared to subjects without AD. Due to PRNT assay validation issues, immunogenicity data from this study were not suitable for inclusion in the package insert or to support vaccine effectiveness or an indication for use specifically in individuals with AD. However, as there is no physiologic reason to suspect decreased effectiveness of JYNNEOS in individuals with AD, there is no reason to specifically exclude (contraindicate) individuals with AD from the general indication for use of this vaccine. The potential benefit of prevention of high risk of mortality (greater than 30%) associated with a smallpox infection outweighs the risks of increased reactogenicity and elevated troponin I levels of uncertain significance in subjects with a history of or active mild atopic dermatitis.

10. CONCLUSIONS

10.1 Vaccine Effectiveness:

1. Effectiveness of MVA-BN has been established in vaccinia naïve subjects by demonstrating non-inferiority of vaccinia specific neutralizing antibody induced by two doses of MVA-BN at days 0 and 28 compared with a single dose of ACAM2000.
2. Non-inferiority comparison of MVA-BN with ACAM2000 was not conducted in populations of AD subjects or HIV-infected subjects because it was not safe to vaccinate these subjects with ACAM2000.
3. Direct comparison of antibody titers following MVA-BN vaccination with titers following ACAM2000 vaccination was not performed among vaccinia-experienced subjects, which is a limitation of this application. Cross-study comparisons of vaccinia specific neutralizing antibody titers are generally not appropriate but, in this case, even more so because great variation exists among the PRNT assays including assay LLOD and LLOQ as well as differences among the study populations. The assay validation issues precluded use of the relevant immunogenicity data to infer effectiveness of a single dose in smallpox vaccine experienced individuals; however, the overall evidence supports inference that the two-dose regimen would be effective in smallpox vaccine experienced individuals, just as in smallpox vaccine naïve individuals.
4. Vaccinia specific neutralizing antibody titers among vaccinia-naïve subjects dropped quickly following primary MVA-BN vaccination series. The antibody titer peaked at 2 weeks after the last dose of primary vaccination (GMT 46) and was almost undetectable at 6 months after the last dose of primary vaccination with a GMT of 7 (assay LLOD ≥6). A single dose of MVA-BN at 2 years after the primary vaccination with MVA-BN induced a booster antibody response. However, the neutralizing antibody titer dropped from a peak GMT of 125 at two weeks after the booster dose to 49 at 6 months after the booster dose. No data were available beyond 6 months after the booster dose. It appears that there may be a need for a booster dose after the primary MVA-BN vaccination. The assay issues from these studies precluded use of the booster dose immunogenicity data to infer effectiveness or optimal timing of a booster dose.
5. Subjects who were vaccinated with the first generation of replicating vaccinia-based smallpox vaccine over 25 years ago also experienced a boost in neutralizing antibody titers after a single dose of MVA-BN. Titers peaked 2 weeks after the booster dose of MVA-BN with a GMT of 175 and reduced to the LLOQ of the assay at 24 months after the booster. The assay issues precluded use of the booster dose immunogenicity data to infer effectiveness or optimal timing of a booster dose for subjects previously vaccinated with replicating vaccinia-based smallpox vaccines.

10.2 Safety

Please refer to Section 8.6 of this review.

11. Risk-Benefit Considerations and Recommendations

11.1 Risk-Benefit Considerations

The risks and benefits analysis of MVA-BN is presented in Table 84 below and discussed in Section 11.2. Although the considerations presented in the table are specific to the smallpox indication, they also generally apply to the monkeypox indication with the following exceptions:

- Although monkeypox is much milder than smallpox, it can be fatal. Typically, case fatality in monkeypox outbreaks has been between 1% and 10%, with most deaths occurring in younger age groups.
- No drugs are currently US-licensed for treatment of monkeypox, and no vaccines (including ACAM2000) are currently US-licensed for prevention of monkeypox.

Table 84: Risk Benefit Assessment for Prevention of Smallpox

Decision Factor	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• Smallpox disease is associated with a fatality rate of 30–40% and severe complications such as keratitis with consecutive blindness, encephalitis, as well as spontaneous abortion and stillbirth in pregnant women • Due to the cessation of smallpox vaccination more than four decades ago, immunity in the general population is low and continuously decreasing, leading to an increasingly susceptible population • Military personnel are at increased risk of terrorist attack with variola	• Smallpox disease is a serious and life-threatening condition based on high fatality rate, associated complications and the potential to weaponize variola virus and use it attack large segments of the population including military personnel.
Unmet Medical Need	• ACAM2000 is a live, vaccinia virus vaccine FDA approved for prevention of smallpox. • Vaccination with ACAM2000 is associated with risks of cardiac events, encephalitis, encephalomyelitis, encephalopathy, progressive vaccinia, generalized vaccinia, severe vaccinial skin infections, erythema multiforme major and eczema vaccinatum. These risks may be increased in subjects with cardiac disease, immune deficiency, or eczema or other skin conditions, and use of ACAM2000 in these populations is limited to situations of high risk for smallpox exposure. ACAM2000 is contraindicated for individuals with severe immunodeficiency. • TPOXX is approved for treatment of smallpox disease via the Animal Rule licensure pathway. There are some uncertainties regarding its effectiveness in humans. • Efficacy of TPOXX is also dependent on initial treatment time point following infection.	• There is an unmet medical need for a smallpox vaccine with an improved safety profile that can be used in a wider population such as subjects with immune deficiencies (such as HIV-infection) or skin diseases (such as atopic dermatitis). • MVA-BN, a non-replicating, vaccinia-based smallpox vaccine, is generally well-tolerated, not associated in clinical trials with the same risk and rates of adverse events as observed with the live vaccine.
Clinical Benefit	• One pivotal trial in vaccinia-naïve healthy subjects 18 through 42 years of age evaluated the effectiveness of MVA-BN by non-inferiority comparison with ACAM2000 in terms of vaccinia specific neutralizing antibodies. The results showed that two doses of MVA-BN administered at 28 days apart was non-inferior to ACAM2000 in eliciting the neutralizing antibodies. • Three clinical trials in subjects previously vaccinated with smallpox vaccines suggested that a single dose of MVA-BN was immunogenic, though assay issues precluded inference of effectiveness. • Two clinical trials in HIV-infected subjects showed that MVA-BN was immunogenic in this population although vaccinia specific neutralizing antibody titers elicited in this population was lower than those in healthy subjects, and assay issues precluded inference of effectiveness. • Vaccinia specific neutralizing antibody titers elicited by MVA-BN in subjects with atopic dermatitis were similar to those in healthy subjects although assay issues precluded inference of effectiveness, and the safety profile in this population was acceptable.	• Two doses of MVA-BN administered at 28 days apart is effective in vaccinia-naïve subjects. • MVA-BN in subjects with atopic dermatitis appears safe, and benefits likely outweigh risks. • MVA-BN appears safe in HIV-infected subjects, and benefits likely outweigh risks. • The available data do not support effectiveness of a single dose of MVA-BN in smallpox vaccine-experienced subjects. There is furthermore insufficient data to support the need and timing for a booster dose in this population.
Risk	• The most common risks of vaccination with MVA-BN are injection-site reactions (pain, erythema, swelling, induration and itching) as well as systemic reactions (myalgia, headache, fatigue and nausea). Most of these reactions are mild in severity, and they resolve relatively quickly and without sequelae. • No safety signals were apparent in the overall study population. Up to 18.4% of subjects in 2 studies developed post-vaccination elevation of troponin. However, all of these troponin elevations were asymptomatic and without a clinically associated event or other sign of myopericarditis.	• No specific clinically relevant, vaccine-related risks have been identified to occur with MVA-BN. • Asymptomatic troponin elevations in 2 of the 22 studies may have been related to the troponin assay used and are of uncertain clinical significance.
Risk Management	• As part of the post-marketing pharmacovigilance plan the applicant has proposed routine collection of cardiac data generated in the standard of care medical care of subjects who receive MVA-BN.	• The planned post-marketing pharmacovigilance plan to further monitor for unanticipated, vaccine-related cardiac events appears adequate.

11.2 Risk-Benefit Summary and Assessment

The data submitted to this application support approval of this BLA. The vaccinia specific neutralizing antibody titer elicited by two doses of MVA-BN administered at 28 days apart in vaccinia naïve individuals was non-inferior to that elicited by ACAM2000. It is reasonable to expect that this regimen of the vaccine is effective in smallpox vaccine naive as well as in smallpox vaccine experienced individuals.

Non-inferiority comparison of MVA-BN with ACAM2000 was not conducted in populations of AD subjects or HIV-infected subjects because it was not safe to vaccinate these subjects with ACAM2000, nor conducted in smallpox-vaccine-experienced subjects. A cross-study comparison of vaccinia specific neutralizing antibody titers is generally not appropriate and in this vaccine development program even more so because great variation exists among the different versions of PRNT including assay LLODs and LLOQs as well as differences among the study populations. These issues preclude a specific inference of effectiveness in HIV-infected subjects or subjects with AD and preclude a determination of whether a single dose of MVA-BN is effective in smallpox vaccine experienced subjects.

The safety profile of MVA-BN is favorable in study subjects including those for whom ACAM2000 is contraindicated or not recommended outside of high risk of smallpox infection, such as HIV-infected subjects or subjects diagnosed with atopic dermatitis. Consequently, the benefit/risk balance is likely to be favorable in all adult populations, and there is no reason to exclude any specific subpopulation from a general indication despite assay issues precluding the labeling of immunogenicity data to infer effectiveness in several of these specific subpopulations.

Reviewer's comment: *The risks and benefits analysis of MVA-BN against smallpox also apply to monkeypox.*

11.3 Discussion of Regulatory Options

Serious issues were identified with regards to the data for ACAM2000 take attenuation in Study POX-MVA-006 and MVA-BN immunogenicity following a single dose in smallpox vaccine experienced subjects and following the two-dose regimen in subjects with HIV infection or atopic dermatitis. Despite these issues, which precluded use of the data to support proposed product labeling for dosing and administration and effectiveness claims in specific subpopulations, other clinical and nonclinical data as described in detail in this review are sufficient to together provide substantial evidence of effectiveness for the two-dose regimen for the prevention of smallpox or monkeypox in all individuals ages 18 years and older. Consequently, approval of the application will be limited to those aspects of the proposed use and labeling that the data support.

11.4 Recommendations on Regulatory Actions

Clinical review of the totality of the safety and immunogenicity data submitted in this application support approval of this BLA to license JYNNEOS for prevention of smallpox or monkeypox in individuals 18 years of age and older.

11.5 Labeling Review and Recommendations

At the time of the completion of this review labeling negotiations with the applicant were still ongoing. The main requested revisions included:

- Removing the take attenuation data from the PI due to concerns with the data quality.
- Removing the immunogenicity data for study POX-MVA-005, -023, -008, -011, and -024 because the PRNT used in these studies were not adequately validated and accepted by CBER assay reviewers.
- Removing the proposed single booster dose for use in smallpox vaccine experienced individuals due to the PRNT assay issues cited above.
- Simplifying the presentation of adverse events by presenting SAE and AESI data of integrated safety summary instead of individual study populations.
- Adding an indication for monkeypox.

11.6 Recommendations on Postmarketing Actions

The applicant has committed to conduct an observational, post-marketing study as part of their routine PVP. The sponsor will collect data on cardiac events that occur and are assessed as a routine part of medical care. Please refer to Dr. Kerry Welsh's review of the PVP for further details.

APPENDICES

Appendix 1: Review of Dose Ranging Studies

The applicant conducted two dose finding studies in the drug development program: POX-MVA-004 and POX-MVA-002.

Study POX-MVA-004

Title of Study: Phase 2, double-blind, randomized, dose-finding study to evaluate
the immunogenicity of three different doses of MVA-BN smallpox vaccine in 18-30 year-old smallpox vaccine naive healthy subjects

Study Period: 14 May 2003 to 14 November 2003

Study Site: Swiss Pharma Contract Ltd, Lettenweg 118, CH-4123 Allschwil, Switzerland

Primary Objective

- To assess the dose of MVA-BN which shows an optimal immunogenicity and reactogenicity profile

Secondary Objectives

- To assess the humoral and cellular immune responses induced to MVA-BN at three different doses
- To assess the safety and reactogenicity of three different doses of the MVA-BN vaccine following repeated vaccination

Study Design

Double-blind, randomized, dose-finding Phase 2 trial in healthy subjects previously not vaccinated against smallpox. The eligible subjects were randomized at 1: 1: 1 into three groups, each receiving two subcutaneous (SC) injections of MVA-BN at indicated doses at 4 weeks apart (at Day 0 and Day 28):

- Group 1: dose 2 x 10^7 $TCID_{50}$, N=55
- Group 2: dose 5 x 10^7 $TCID_{50}$, N=55
- Group 3: dose 1 x 10^8 $TCID_{50}$, N=54

Solicited adverse events were collected for 7 days after each vaccination. Unsolicited adverse events were followed for 30 days after each vaccination and SAEs were followed up for 8 weeks after the last vaccination. Immune responses determined by MVA based ELISA and PRNT (refer to Section 9.5.2.1, page 33, CSR of POX-MVA-004) were assessed at baseline (Day 0), two weeks after the first dose (Day 28), two weeks after the second dose (Day 42) and at the end of the study (Day 84).

Results of Immune Responses

Immune responses following MVA-BN vaccinations were assessed using MVA specific ELISA and PRNT assays and analyzed on the per protocol set (PPS) population. The PPS was defined as subjects who had received all study vaccinations, were seronegative for anti-MVA ELISA (titer <50) at baseline and had no major protocol violation.

The primary endpoint of the study was seroconversion rate determined by ELISA. Seroconversion determined by ELISA was defined as ELISA GMT ≥1: 50. At Day 28 after the first vaccination, 59.3%, 81.6% and 94.2% of the subjects were seroconverted in Groups 1,2 and 3, respectively. At two weeks after the second vaccination (Day 42), all subjects of all Groups were seroconverted. Two months after the last vaccination (Day 84), 94.4% of the subjects in Group 1, and 100% of the subjects in Group 2 and Group 3 were seropositive.

The antibody titers determined by both ELISA and PRNT are presented in Table 1. As seen from Table 1, a dose dependent antibody response determined by both ELISA and PRNT was observed. Peak antibody titers were observed at two weeks after the second vaccination regardless of vaccine dose level and immunoassay used.

Table 1: Summary of Humoral Immune Responses Determined by ELISA and PRNT (PPS)

Group	Time Point	ELISA GMT (95% CI)	PRNT GMT (95% CI)
Group 1 (MVA 2 x 10^7 $TCID_{50}$) (N=54)	Day 0	1.0 (NA, NA)	1.0 (NA, NA)
	Day 28	14.4 (7.7, 26.8)	1.3 (1.0, 1.7)
	Day 42	377.2 (288.3, 493.5)	5.5 (3.2, 9.6)
	Day 84	134.3 (91.1, 198.2)	1.9 (1.3, 2.9)
Group 2 (MVA 5 x 10^7 $TCID_{50}$) (N=54)	Day 0	1.0 (NA, NA)	1.0 (NA, NA)
	Day 28	53.3 (29.9, 94.9)	1.6 (1.1, 2.2)
	Day 42	583.6 (461.6, 737.9)	10.3 (5.8, 18.4)
	Day 84	227.8 (176.4, 294.1)	2.3 (1.5, 3.7)
Group 3 (MVA 1 x 10^8 $TCID_{50}$) (N=54)	Day 0	1.0 (NA, NA)	1.0 (NA, NA)
	Day 28	98.5 (67.6, 143.7)	1.5 (1.1, 1.9)
	Day 42	813.7 (628.7, 1053.3)	19.4 (11.1, 34.2)
	Day 84	323.6 (246.8, 424.3)	2.9 (1.8, 4.8)

Source: Adapted from Table 23 (page 57) and Table 32 (page 66), POX-MVA-004 CSR, Module 5.3.4.1, STN125678/0.
Note: ELISA assay cut-off value of 1:50; PRNT assay cut-off value, 1:20. NA=not applicable

Reviewer's comment: *PRNT assay was less sensitive as compared with ELISA. Only 71.2% of subjects in the highest dose group were seropositive at peak visit (two weeks after the second dose) based on the PRNT assay (i.e., PRNT titer >1: 20).*

Safety Results

Overview of solicited injection-site and systemic adverse events are presented in Table 2. The most common injection-site reactions were injection-site pain and erythema, and the most common systemic reactions were headache and fatigue. In general, both injection-site and systemic adverse reactions across the three dose groups were similar in terms of incidence rates and intensity except for injection-site erythema and swelling. The proportions of subjects with inject-site erythema and swelling were numerically higher in the higher dose groups. Most solicited reactions resolved in 3-4 days.

Table 2: Summary of Solicited Injection-Site Reactions and Systemic Reactions Reported in the Seven Day Follow-Up Period After Any Vaccination (FAS Population)

Adverse Event	**Group 1 (N=55) n (%)**	**Group 2 (N=55) n (%)**	**Group 3 (N=54) n (%)**
Injection-Site Reactions	**52 (94.5)**	**50 (90.9)**	**53 (98.1)**
Any pain	48 (87.3)	44 (80.0)	53 (98.1)
Grade 3 pain	0 (0.0)	3 (5.5)	2 (3.7)
Any erythema	47 (85.5)	49 (89.1)	52 (96.3)
Erythema ≥50 mm	7 (12.7)	13 (23.6)	15 (27.8)
Any swelling	38 (69.1)	41 (74.5)	44 (81.5)
Swelling ≥50 mm	4 (7.3)	7 (12.7)	9 (16.7)
Any induration	43 (78.2)	41 (74.5)	43 (79.6)
Induration ≥50 mm	3 (5.5)	6 (10.9)	5 (9.3)
Systemic Reactions	**37 (67.3)**	**33 (60.0)**	**30 (55.6)**
Any pyrexia	0 (0.0)	2 (1.8)	2 (1.9)
Any headache	39 (35.5)	35 (31.8)	33 (30.6)
Grade 3 headache	4 (3.6)	2 (1.8)	3 (2.8)
Any myalgia	14 (12.7)	12 (10.9)	19 (17.6)
Grade 3 myalgia	1 (0.9)	1 (0.9)	0 (0.0)
Any nausea	12 (10.9)	11 (10.0)	17 (15.7)
Grade 3 nausea	1 (0.9)	2 (1.8)	1 (0.9)
Any fatigue	39 (35.5)	30 (27.3)	42 (38.9)
Grade 3 fatigue	1 (0.9)	3 (2.7)	2 (1.9)

Source: Adapted from Table 48 (page 83), and Table 51 (page 88) and Table 54 (page 91), POX-MVA-004 CSR, Module 5.3.4.1, STN125678/0.

No treatment related SAEs were reported from the study.

Conclusion

The study demonstrated that immunization with MVA-BN induced a dose dependent antibody response. Peak antibody response was identified at two weeks after the second dose regardless of the dose level of MVA-BN.

The safety profile for all the three dose levels was acceptable. Incidence of injection-site erythema and swelling were numerically higher at higher dose-levels, while incidence of systemic reactions did not show a clear dose response. No treatment related SAE were reported with any dose level.

Study POX-MVA-002

Title of Study: A Phase 1 Clinical Trial to Evaluate the Safety and Immunogenicity of MVA-BN in a Dose Response Regimen Followed by Administration of Dryvax in Healthy Adult Subjects

Study Period: 10 May 2004 to 06 June 2006

Study Center: Saint Louis University, Vaccine and Treatment Evaluation Unit

Objective

- To assess the safety and tolerability of MVA-BN
- To evaluate the ability of different doses of MVA-BN to induce humoral immune responses

Study Design

This was a randomized, double-blind, dose-response study to assess the safety and immunogenicity of MVA-BN vaccination in healthy, previously smallpox vaccine unvaccinated subjects. Each subject was randomly assigned to 1 of 6 groups (A to F), each receiving 2 injections of MVA-BN or placebo separated by a 4-week interval (at Day 0 and Day 28) followed by a single dose of Dryvax or placebo at Day 112. Dryvax was administered by scarification with a bifurcated needle in a dose of approximately 0.0025 mL of 1 X 10^8 PFU/mL or about 10^5 PFU/dose. All vaccines were administered by an unblinded vaccinator who did not have subsequent contact with study subjects. The treatment groups were as follows:

- Group A (n =15): MVA-BN ($2x10^7$ tissue culture infective dose50 [TCID50]): SC, at Day 0 and Day 28; Dryvax at Day 112
- Group B (n =15): MVA-BN ($5x10^7$ $TCID_{50}$), SC, at Day 0 and Day 28; Dryvax at Day 112
- Group C (n =15): MVA-BN ($1x10^8$ $TCID_{50}$), SC, at Day 0 and Day 28; Dryvax at Day 112
- Group D (n =15): Placebo, SC, at Day 0 and Day 28; Dryvax at Day 112
- Group E (n =15): MVA-BN ($1x10^8$ $TCID_{50}$), SC, at Day 0 and Day 28; Placebo at Day 112
- Group F (n =15): MVA-BN ($1x10^8$ $TCID_{50}$), IM, at Day 0 and Day 28; Dryvax at Day 112

The safety assessments included local and systemic reactogenicity in 15 days following each vaccination, and SAEs throughout the study up to six months after the last vaccination.

Immunogenicity evaluations included vaccinia neutralizing antibody as assessed by PRNT and binding antibody to vaccinia as assessed by ELISA.

Efficacy of Dryvax was evaluated by the incidence of takes, defined as a lesion at the injection site consistent with the Jennerian process.

Immunogenicity Results

Vaccination of study subjects with various doses of MVA-BN (Group A: 2 x 10^7, Group B: 5 x 10^7 and Group C: 1 x 10^8 $TCID_{50}$) SC elicited dose-dependent vaccinia specific neutralizing antibody response determined by both Dryvax and MVA based PRNT assays. The geometric mean antibody titers (GMT) at two weeks after the second dose (Day 42) for Groups A, B and C were 72.1, 143.5 and 182.1 as determined by Dryvax based PRNT respectively, and 347.2, 551.5 and 914.5 as determined by MVA based PRNT respectively (Table 3).

MVA-BN administered IM elicited the similar antibody responses as MVA-BN administered SC. For example, Dryvax based PRNT GMTs at Day 42 for MVA-BN administered SC (Group C) and IM (Group F) were 182.1 and 179.9, respectively. Similarly, MVA based PRNT GMTs at Day 42 for MVA-BN administered SC and IM were 914.5 and 748.8, respectively (Table 3).

Similar results were obtained when vaccinia specific antibodies were determined by ELISA (Data not shown).

Table 3: Summary of Neutralizing Antibody Responses at Various Time Points after Each Vaccination Determined by Various Vaccinia Virus Strain Based PRNT

Group	Time Point	Dryvax PRNT GMT (95% CI)	MVA PRNT GMT (95% CI)	VV-WR PRNT GMT (95% CI)
A: 2 x 10^7 $TCID_{50}$, SC MVA/MVA/Dryvax (N=15)	Day 0	3.0 (2.2, 4.1)	2.2 (1.8, 2.7)	No Data
	Day 14	22.5 (15.8, 31.9)	24.9 (14.4, 42.9)	
	Day 28	11.6 (7.4, 18.1)	13.7 (7.0, 27.0)	
	Day 42	72.1 (41.9, 124.0)	347.2 (161.9, 744.7)	
	Day 56	37.1 (17.2, 80.1)	128.3 (63.7, 258.2)	
	Day 112	20.7 (11.1, 38.7)	50.1 (20.2, 124.6)	
	Day 140	258.9 (121.6, 551.2)	451.6 (240.6, 847.7)	
	Day 184	128.1 (57.5, 285.4)	288.9 (129.5, 644.6)	
B: 5 x 10^7 $TCID_{50}$, SC MVA/MVA/Dryvax (N=15)	Day 0	3.2 (1.8, ,5.5)	2.1 (1.9, 2.4)	No Data
	Day 14	25.0 (14.0, 44.6)	28.8 (13.7, 60.3)	
	Day 28	16.9 (9.3, 30.5))	23.8 (12.5, 45.2)	
	Day 42	143.5 (80.8, 255.0)	551.5 (321.5, 946.0)	
	Day 56	99.1 (66.7, 147.3)	309.2 (162.2, 589.3)	
	Day 112	37.1 (19.6, 70.3)	132.9 (70.1, 251.9)	
	Day 140	165.2 (66.3, 411.5)	377.4 (203.6, 699.9)	
	Day 184	93.8 (44.7, 196.8)	190.3 (96.3, 376.1)	
C: 1 x 10^8 $TCID_{50}$, SC MVA/MVA/Dryvax (N=15)	Day 0	3.6 (2.3, 5.6)	2.4 (1.6, 3.4)	NA
	Day 14	40.2 (25.6, 62.9)	51.0 (23.7, 110)	NA
	Day 28	22.3 (13.9, 36.0)	26.3 (12.3, 56.5)	3.8 (NA)
	Day 42	182.1 (84.9, 390.3)	914.5 (528.0, 1584)	107.7 (NA)
	Day 56	96.4 (49.8, 186.6)	468.9 (254.8, 863.1)	NA
	Day 112	56.0 (32.3, 97.1)	182.1 (95.9, 346.0)	NA
	Day 140	273.5 (139.7, 535.3)	614.5 (276.2, 1367)	193.2 (NA)
	Day 184	130.9 (78.3, 218.9)	344.7 (211.1, 562.9)	NA
D: Placebo, SC Placebo/Placebo/Dryvax (N=15)	Day 0	3.8 (2.5, 5.5)	2.2 (1.8, 2.7)	NA
	Day 14	4.1 (2.7, 6.3)	2.0 (NA, NA)	NA
	Day 28	4.1 (2.7, 6.3)	2.0 (NA, NA)	NA
	Day 42	4.5 (2.9, 6.8)	2.5 (1.5, 4.1)	NA
	Day 56	4.1 (2.7, 6.4)	2.5 (1.6, 3.9)	NA
	Day 112	5.4 (2.9, 9.9)	2.2 (1.8, 2.7)	149.3 (NA)
	Day 140	691.8 (371.1, 1290)	110.2 (38.8, 313.2)	NA
	Day 184	316.2 (191.8, 521.1)	73.8 (34.3, 158.7)	
E: 1 x 10^8 $TCID_{50}$, SC MVA/MVA/Placebo (N=15)	Day 0	3.7 (2.3, 5.8)	2.0 (NA, NA)	NA
	Day 14	58.9 (38.4, 90.4)	89.4 (50.1, 159.5)	7.5 (NA)
	Day 28	26.0 (14.9, 45.3)	42.9 (22.4, 82.2)	86.0 (NA)
	Day 42	130.5 (74.0, 230.1)	619.4 (248.1, 1547)	NA
	Day 56	97.4 (52.5, 180.6)	285.4 (137.4, 592.7)	NA
	Day 112	64.8 (46.6, 90.2)	194.7 (128.1, 296.0)	38.7 (NA)
	Day 140	39.0 (29.4, 51.8)	127.33 95.09 170.51	NA
	Day 184	48.9 (25.5, 93.5)	147.7 (86.1, 253.1)	
F: 1 x 10^8 $TCID_{50}$, IM MVA/MVA/Dryvax (N=15)	Day 0	4.4 (2.5, 7.7)	2.0 (NA, NA)	No Data
	Day 14	34.7 (22.5, 53.3)	47.0 (24.6, 89.7)	
	Day 28	26.1 (18.4, 36.8)	27.8 (12.8, 60.3)	
	Day 42	179.9 (99.9, 323.8)	748.8 (380.2, 1475)	
	Day 56	101.2 (55.3, 185.6)	463.2 (268.3, 799.7)	
	Day 112	73.7 (42.1, 128.8)	217.7 (107.0, 442.9)	
	Day 140	250.0 (112.2, 556.5)	689.3 (306.9, 1548)	
	Day 184	122.0 (62.3, 238.7)	328.1 (192.9, 558.1)	

Source: Adapted from Table 2 (page 24-25) and Table 3 (page 25), Response to IR#8, Module 1.11.3, STN125678/0.9
Note: PRNT assay cut-off value=1:20. SC=subcutaneous injection' IM=intramuscular injection. NA=Not applicable. MVA/MVA/Dryvax, or MVA/MVA/Placebo, or Placebo/Placebo /Dryvax = first/second/third vaccination. The first, second and third vaccination was given at Day 0, 28 and 112, respectively. The dose provided in each group was for MVA-BN only.

Reviewer's comment: *It is not unexpected that antibody titers determined by PRNT using different reporter viruses varied greatly. It appears that virus strain used in previous vaccination would have significant impact on antibody titers, depending the virus strain used in the PRNT assay, elicited by vaccination with a different virus strain. As seen from Table 4, peak antibody titers increased after the second dose of MVA-BN, and increased further after a booster with Dryvax, if antibody titers were determined by Dryvax based PRNT. However, compared to the peak titer after the second dose of MVA-BN, antibody titer decreased following a booster with Dryvax if antibody titer was determined by MVA based PRNT.*

Table 4: Comparison of Anti-Vaccinia Neutralizing Antibody following Vaccination with MVA-BN and Dryvax Determined by PRNT Using Dryvax, MVA-BN and VV-WR as a Report Virus

	PRNT-Dryvax	PRNT-MVA	PRNT-WR
Group C: Titer at Day 14 (Peak titer after 1st doses of MVA-BN)	40	51	No data
Group C: Titer at Day 42 (Peak titer after 2nd doses of MVA-BN)	182	915	107
Group C: Titer at Day 140 (Peak titer after a single dose of Dryvax)	273	614	193
Group D: Titer at Day 140 (Peak titer after a single dose of Dryvax in vaccinia naïve Subjects)	692	110	149

Source: Adapted from Table 1-3, Module 1.11.3_Responses to IR8, STN125678/0.9

Clinical Take Results

Take rates and skin lesion sizes were assessed for groups in which subjects were vaccinated with Dryvax (Groups A, B, C, D and F). Take rates are presented in Table 5. In comparing the combined groups vaccinated with MVA-BN regardless of dose and route (Group A, B, C and F) with subjects received placebo (Group D), there was no significant difference in the take rate (p=0.10) after Dryvax challenge.

Table 5: Take Rates Following Vaccination with Groups in POX-MVA-002

Group	N	n	Take Rate (95% CI)
A: 2 x 10^7 $TCID_{50}$, SC (MVA/MVA/Dryvax)	14	13	92.9 (66.1, 99.8)
B: 5 x 10^7 $TCID_{50}$, SC (MVA/MVA/Dryvax)	13	7	53.8 (25.1, 80.8)
C: 1 x 10^8 $TCID_{50}$, SC (MVA/MVA/Dryvax)	11	10	90.9 (58.7, 99.8)
D: Placebo, SC (Placebo/Placebo/Dryvax)	13	13	100 (75.3, 100)
F: 1 x 10^8 $TCID_{50}$, IM (MVA/MVA/Dryvax)	12	8	66.7 (34.9, 90.1)

Source: Adapted from Table 7 (page 30), Module 1.11.3_Responses to IR8, STN125678/0.9
Note: N=number of subjects with data available; n= number of subjects with take.

The lesion size at various time points following Dryvax vaccination was assessed. The lesion sizes at Day 6-8 and at any day after Dryvax vaccination are presented in Table 6. Vaccination with MVA-BN significantly reduced the size of lesion caused by Dryvax vaccination. The degree of take attenuation does not appear to be dependent on MVA-BN dose, route of administration or neutralizing antibody titer prior to Dryvax vaccination (Table 6). For subjects who received the to-be-licensed regimen (i.e., Group C), there were 17.7% and 38.3% reduction in lesion size at Day 6-8 and at Any Day, respectively, comparing to subjects who were vaccinia naïve prior to Dryvax vaccination (Group D).

Table 6: Summary of Skin Lesion Size (mm) Following Vaccination with Dryvax

Group	PRNT GMT at Day 112	Lesion Size (Day 6-8) Mean ± SD	Lesion Size (Any Day) Mean ± SD
A: 2 x 10^7 $TCID_{50}$, SC MVA/MVA/Dryvax N=13	Dryvax based: 20 MVA based: 50	6.2 ± 2.3	8.2 ± 3.0
B: 5 x 10^7 $TCID_{50}$, SC MVA/MVA/Dryvax N=7	Dryvax based: 37 MVA based: 133	6.3 ± 2.9	8.1 ± 3.6
C: 1 x 10^8 $TCID_{50}$, SC MVA/MVA/Dryvax N=10	Dryvax based: 56 MVA based: 182	6.5 ± 2.2	8.7 ± 3.3
D: Placebo, SC Placebo/Placebo/Dryvax N=13	Dryvax based: 5 MVA based: 2	7.9 ± 2.6	14.1 ± 3.3
F: 1 x 10^8 $TCID_{50}$, IM MVA/MVA/Dryvax N=8	Dryvax based: 65 MVA based: 195	6.6 ± 3.2	7.5 ± 3.3

Source: Adapted from Table 8 (page 31-33), Module 1.11.3_Responses to IR8, STN125678/0.9
Note: N=number of subjects with take.

Safety Results

Injection-site reactions after MVA-BN

There were no statistically significant differences in injection-site reactions among groups who received MVA-BN subcutaneously (Groups A, B, C, and E). Significant differences in injection-site reactions were found between the MVA-BN IM (Group F) and the high dose MVA-BN SC (Group C) groups. Significant differences were observed in rates of any local reactions between the highest SC MVA-BN dose group (Group C) and the lowest MVA-BN dose (Group A).

Systemic symptoms after MVA-BN

There were no significant differences observed among groups after any dose of MVA-BN for any systemic symptom.

Conclusions

The safety profile of MVA-BN was acceptable. Subcutaneous administration of MVA-BN appeared to be less reactogenic than intramuscular administration.

MVA-BN was immunogenic at doses of 2×10^7 to 1×10^8 $TCID_{50}$ with no significant differences between the SC or IM administration route. Two doses of MVA-BN resulted in a dose-dependent antibody response as determined by ELISA and PRNT.

Previous immunization with MVA-BN did not appear to reduce the occurrence of take rates following Dryvax vaccination, but significantly reduced the size of cutaneous lesions after Dryvax vaccination.