STATISTICAL ANALYSIS PLAN (SAP)

21 March 2016

FY12-19, HP-12-19, POX-MVA-006
A randomized, open-label Phase III non-inferiority trial to compare indicators of efficacy for MVA BN® smallpox vaccine to ACAM2000® in 18-42 year old healthy vaccinia-naïve subjects

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List of Abbreviations

AAR	Area Attenuation Ratio
ACV	Assay Cut-off Value
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ATC	Anatomical-Therapeutical-Chemical
BMI	Body Mass Index
BN	Bavarian Nordic
CBC	Complete Blood Count
CI	Confidence Interval
CTP	Clinical Trial Protocol
DAR	Diameter Attenuation Ratio
DRM	Data Review Meeting
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
eCRF	electronic Case Report Form
ELISA	Enzyme-linked Immunosorbent Assay
FAS	Full Analysis Set
FU	Follow-up
GMT	Geometric Mean Titer
HL	Hodges-Lehmann
IMLD	Investigator Measured MLD
ISS	Initially Seronegative Subset
ITRC	Independent Take Review Committee
LCL	Lower Confidence Limit
MedDRA	Medical Dictionary for Regulatory Activities
MLA	Maximum Lesion Area
MLD	Maximum Lesion Diameter
MMLD	Memory aid MLD
MVA-BN®	Modified Vaccinia Ankara – Bavarian Nordic
PB	Price-Bonett
PPS	Per Protocol Set
PRNT	Plaque Reduction Neutralization Test
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis Systems
s.c.	Subcutaneous
SCR	Screening Visit
SD	Standard Deviation
SMLD	SilhouetteConnect camera system MLD
SOC	System Organ Class
SOP	Standard Operating Procedure

$TCID_{50}$	Tissue Culture Infectious Dose 50%
V	Visit
WHO-DD	Word Health Organization Drug Dictionary

Statistical Analysis Plan

This Statistical Analysis Plan (SAP) outlines the contents and methods to be used for the statistical analysis of the data collected during clinical trial POX-MVA-006 (FY12-19, HP-12-19) for all subjects. Prior to database lock a final version of the SAP will be produced.

This document is intended to provide additional information to the Clinical Trial Protocol (CTP). In case of a revision of the CTP the SAP will only be amended if there is a direct impact on the SAP.

Changes from Edition 7.0 to 8.0 of the SAP: the Hodges-Lehmann (HL) method of calculating the confidence interval of the ratio of the Maximum Lesion Area (MLA) medians replaces the Price-Bonett (PB) method in the analysis of the MLA-specific primary endpoint. The PB based calculation method will nevertheless be used for supportive secondary analysis of the MLA endpoint.

General Definitions

Full Take:

A full take is a successful primary take according to Vaccinia (Smallpox) Vaccine Recommendations of the Advisory Committee on Immunization Practices (ACIP) (Morbidity and Mortality Weekly Report [MMWR] June 22, 2001).

Formation of a major cutaneous reaction at the ACAM2000® vaccination site:

- The inoculation site becomes reddened and pruritic three to four days after vaccination.
- A vesicle surrounded by a red areola then forms, which becomes umbilicated (collapsed center) and then pustular by days seven to eleven after vaccination.
- The pustule begins to dry; the redness subsides; and the lesion becomes crusted between the second and third week, the scab falls off, leaving a permanent scar that at first is pink in color but eventually becomes flesh colored.

Partial Take:

After revaccination, skin reactions at the ACAM2000® vaccination site might be less pronounced with more rapid progression and healing than those after primary vaccination. Revaccination is successful if a pustular lesion or area of definite induration or congestion surrounding a central lesion (i.e. scab or ulcer) appears six to eight days after re-vaccination. A partial take is a successful primary take.

The above definitions of Take are according to Vaccinia (Smallpox) Vaccine Recommendations of the Advisory Committee on Immunization Practices (ACIP) (Morbidity and Mortality Weekly Report [MMWR] June 22, 2001).

Absent Take:

An absent take is considered to be the absence of a cutaneous reaction i.e. consistent with a full or partial take, at the ACAM2000® vaccination site.

Attenuation:

After revaccination, skin reactions at the ACAM2000® vaccination site are often less pronounced and there is more rapid progression and healing than those after primary vaccination. For the purposes of this SAP and corresponding CTP attenuation is considered to be when the takes observed in Group 1 subjects (MVA-BN® recipients vaccinated with ACAM2000®) are determined to be diminished full takes based on the median of the individually measured parameter of Maximum Lesion Areas in Group 1 subjects, in comparison to the median of Maximum Lesion Areas of takes in Group 2 subjects (ACAM2000® recipients).

Peak Visit:

For the purposes of this SAP, the Peak Visit is the visit with the highest expected antibody titers. For MVA-BN® the peak responses are considered to be at Day 42 after the first MVA-BN® vaccination (as described in the MVA-BN® Investigator's Brochure) when the subject has received two (2) MVA-BN® vaccinations according to the standard vaccination schedule. For ACAM2000® the peak responses are considered to be at Day 28 post vaccination in order to be in alignment with published data for conventional smallpox vaccines reaching a peak four weeks post vaccination (Frey, 2003; Belshe, 2004; Kennedy, 2004; Frey, 2007), which is also in full agreement with the data published for ACAM2000® (ACAM2000 Vaccines and Related Biological Products Advisory Committee [VRBPAC] Briefing Document, April 2007).

Individual Peak Titer:

For each subject in Group 1 the individual maximum Enzyme-linked Immunosorbent Assay (ELISA) titer from Visit 1 to Visit 7 will be used as the individual peak ELISA titer, and in Group 2 the individual maximum ELISA titer from Visit 1 to Visit 6 will be used. The individual peak Plaque Reduction Neutralization Test (PRNT) titer is then defined in the same way. Here it should be noted that although the visit numbering is a little different between groups the serum sample are taken at the same time points for Group 1 and Group 2 (no sample is taken at Visit 5 in Group 1).

Enrolled Subjects:

Subjects are considered enrolled once they have received the first trial vaccination. For this trial a vaccination means administration of either MVA-BN® and/or ACAM2000®.

Trial Day:

The day of trial is calculated from the day of the first vaccination. The day of first vaccination is defined as Day 0 and the day after first vaccination is Day 1, and so on. Equally, the day before

first vaccination is defined as Day -1, and so on. In particular, no reference is made to the time of the vaccination in the calculation of the trial day, i.e. at midnight a new trial day begins regardless of the time of first vaccination. Note that it is possible for some Visit 1 procedures to be performed, for example, on trial Day -1 prior to vaccination at Day 0.

Baseline:

If not otherwise specified, 'Baseline' refers to the last measurement before first vaccination of trial drug. This is either at Visit 1 or Screening (SCR), or latest re-screening as appropriate. If there is missing vital sign data at Visit 1 then the Screening Visit or latest re-screening data will be used to impute the missing data. These data will be summarized as Baseline data without direct specification of which visit was used. However, at all other visits no imputation will be used and the actual visit used will be specified in tables or listings.

Screening Phase:

The screening phase is defined from the Screening Visit up to Visit 1 before the first vaccination. This includes any re-screening visits that are conducted.

Active Trial Phase:

All data collected from the first vaccination at Visit 1 up to (and including) either Visit 10 for Group 1 or Visit 4 for Group 2, or trial early termination visit. This could include any unscheduled visits up until Visit 10 or Visit 4, as appropriate.

FU Trial Phase:

The follow-up period begins immediately after the active trial phase and continues until the (remote) FU (Follow-up) Visit.

All data collected after Visit 10 for Group 1 and Visit 4 for Group 2 until, and including, the remote FU Visit (and if performed on-site FU Visit). Note that for some subjects for whom the remote FU Visit was not performed there may not be any FU phase data available, however this will not be considered a protocol deviation.

Eligible Subjects:

A subject is eligible if the question "is the subject eligible for the trial?" is answered with "yes" and there are no subsequent major protocol deviations that affect the eligibility of the subject.

Subjects Receiving Vaccinations:

A subject received trial vaccination according to the randomized group assignment if a date of drug administration is given for the vaccination and no major vaccine handling deviation is recorded on the electronic Case Report Form (eCRF).

Treatment Emergent Adverse Event:

An adverse event (AE) with onset either on the day of a vaccination, but after the vaccination, or within the 28 days following a vaccination.

Causally Related Adverse Event:

An AE with either a 'possible', 'probable', 'definite', 'unknown' or 'not evaluable' or missing relationship to the vaccine.

An adverse event with missing relationship and onset before the first vaccination will not be assumed to be causally related.

Adverse Drug Reaction:

A causally related treatment emergent AE.

Serious Adverse Event:

All Serious Adverse Events (SAE) recorded with onset after the first trial vaccination and before the end of the FU phase are considered as treatment emergent regardless of the day of onset.

Adverse Events of Special Interest:

Any Adverse Event of Special Interest (AESI) which is also classified as serious will be included in the analysis of SAEs and will not be included in the analysis of AESIs. All AESIs recorded with onset after the first trial vaccination and before the end of the FU phase are considered as treatment emergent regardless of the day of onset.

Assay Cut-off Value:

The Assay Cut-off value (ACV) for the vaccinia-specific ELISA can be found in SOP BN0002809: "Automated ELISA for Detection of Vaccinia Specific Antibodies in Human Sera", and for the vaccinia-specific PRNT can be found in SOP BN0003536: "Human Plaque Reduction Neutralization Test Using Vaccinia Virus Western Reserve".

The Geometric Mean Titer:

The Geometric Mean Titer (GMT) is calculated by taking the antilogarithm of the mean of the $\log_{10}$ transformed titers. Antibody titers below the ACV will be given an arbitrary value of one for the purpose of this calculation.

Seronegative and Seropositive Result:

A seronegative result is a titer below the ACV, while a seropositive result is a titer equal to or above the ACV.

Seroconversion:

Seroconversion is defined at each post baseline visit as either the appearance of antibody titers ≥ ACV for initially seronegative subjects or a doubling or more of the antibody titer compared to baseline titer at Visit 1 for initially seropositive subjects. Seroconversion is not calculated if either the Visit 1 or respective post baseline visit titers are missing.

Maximum Lesion Area:

The Maximum Lesion Area (MLA) is defined as the maximum of two measurements: the lesion area measured on Day 6-8 (after scarification) or the lesion area measured on Day 13-15 (after scarification).

There is only one lesion area measurement performed using the SilhouetteConnect camera system.

If one of the lesion areas is missing, then the MLA will be calculated as the single lesion area which is present. However, subjects with only one lesion area recorded will be considered a major protocol violation and will be excluded from the per protocol set (and therefore also from the initially seronegative set).

The MLA will be used in the co-primary endpoint analysis. However, the Day 6-8 and Day 13-15 lesion areas will also be reported and included as descriptive secondary endpoints.

Maximum Lesion Diameter:

The Maximum Lesion Diameter (MLD) is the largest major diameter measured across the lesion on Day 6-8 (after scarification) or Day 13-15 (after scarification).

There are three separate measurements of the MLD

- The SilhouetteConnect camera system MLD (SMLD) recorded on Day 6-8 (after scarification) or Day 13-15 (after scarification)
- The Investigator Measured MLD (IMLD) recorded on Day 6-8 (after scarification) or Day 13-15 (after scarification)
- The Memory aid MLD (MMLD) recorded by the subject on each day after scarification.

The Investigator maximum diameter, Day 6-8 diameter and Day 13-15 diameters will be used as supportive secondary analyses.

Lesion:

Lesion measurements (area and diameter) referred to in this SAP will include signs (after scarification) of pustule, vesicle, pus, ulcer or scab but will not include extended symptoms of induration or erythema.

1 Trial Overview

1.1 Trial Description

This is a randomized, open-label Phase III non-inferiority trial to compare indicators of efficacy for MVA-BN® smallpox vaccine to ACAM2000® in 18-42 year old healthy vaccinia-naïve subjects.

A total of 440 vaccinia-naive subjects will be assigned randomly to two parallel treatment groups in a 1:1 ratio, 220 subjects in Group 1 and 220 subjects in Group 2.

Group 1:

One 0.5 ml standard dose MVA-BN® liquid-frozen vaccine contains a nominal titer of $1x10^8$ tissue culture infectious dose 50% ($TCID_{50}$) Modified Vaccinia Ankara – Bavarian Nordic (MVA-BN®).

Two vaccinations each of 0.5 ml MVA-BN® vaccine will be administered four weeks apart (Day 0 and Day 28) as a subcutaneous (s.c.) injection in the non-dominant upper arm, followed by a single vaccination of ACAM2000® administered four weeks after the second MVA-BN® vaccination (Day 56). As per the prescribing information: one dose of reconstituted ACAM2000® vaccine consists of $2.5\text{-}12.5x10^5$ plaque forming units of live vaccinia virus. A droplet (~ 0.0025 ml) vaccine is picked up with a bifurcated needle and is administered by the percutaneous route (scarification) using 15 jabs of the bifurcated needle.

Group 2:

A single vaccination of ACAM2000® will be administered at Day 0.

The active phase of the trial will begin at Day 0 (Visit 1) and continue through the second MVA-BN® vaccination (Day 56) and until 28 days after the ACAM2000® vaccination of Group 1 subjects (Visit 10). The active phase of the trial will begin at Day 0 (Visit 1) and continue until 28 days after the ACAM2000® vaccination of Group 2 subjects (Visit 4).

A remote Follow-up (FU) Visit by telephone or email is planned six months after the last trial vaccination. Only if there is a medical need, will the subject be asked to come back to the site or at the FU Visit (and specifically in the case of deployed military subjects) at another medical unit as agreed upon by the Principle Investigator.

The total duration (including screening period) is up to 22 weeks for Group 1 and up to 18 weeks for Group 2, plus remote correspondence (e.g. a phone call or email) six months after the last vaccination. If the remote correspondence reveals a medical need or any underlying condition that requires further examinations, the subject will be called in for a physical visit.

1.2 Objectives of the Trial

1.2.1 Co-Primary Objectives

To demonstrate the efficacy of MVA-BN® by assessing non-inferiority of MVA-BN® compared to ACAM2000® in terms of vaccinia-specific Plaque Reduction Neutralization Test (PRNT) antibody response 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 administration of ACAM2000® results in an attenuation of take in terms of MLA.

1.2.2 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 ACAM2000® given alone or ACAM2000® given after MVA-BN® priming.

1.3 Trial Population

Four hundred and forty (440) male and female volunteers from military personnel, aged 18 to 42 years who meet all of the inclusion and none of the exclusion criteria will be recruited for enrollment into this trial.

1.4 Endpoints

1.4.1 Co-Primary Endpoints

- PRNT GMT at the Peak Visits
- Maximum Lesion Area (MLA) in mm^2 after scarification with ACAM2000®

Both co-primary endpoint analyses will be conducted using the Per Protocol Set (PPS). However, identical secondary robustness analyses will also be conducted using the Full Analysis Set (FAS) and the Initially Seronegative Subset (ISS), a subset of the PPS.

1.4.2 Secondary Immunogenicity Endpoints

- GMTs at the Peak Visits measured by vaccinia-specific ELISA.
- GMTs at all antibody blood sample time points measured by vaccinia-specific PRNT and vaccinia-specific ELISA.

- PRNT seroconversion rates at Peak Visits defined as the percentage of initially seronegative subjects with appearance of antibody titers equal or greater than the ACV in a vaccinia-specific PRNT.
- ELISA seroconversion rates at Peak Visits defined as the percentage of initially seronegative subjects with appearance of antibody titers equal or greater than the ACV in a vaccinia-specific ELISA.

These secondary analyses will be conducted on the PPS, FAS and ISS.

1.4.3 Secondary Efficacy Endpoints

- Investigator assessed Maximum Lesion Diameter (MLD) in mm after scarification with ACAM2000®
- Investigator assessed Lesion diameter in mm at Day 6-8 after scarification with ACAM2000®
- Investigator assessed Lesion diameter in mm at Day 13-15 after scarification with ACAM2000®
- The individual take will be classified as either full or partial or absent by a blinded Independent Take Review Committee (ITRC), (see ITRC Charter Edition 3 dated 27-Feb-2015 for details).
- Lesion area in mm^2 at Day 6-8 after scarification with ACAM2000®
- Lesion area in mm^2 at Day 13-15 after scarification with ACAM2000®

(The correct measurement of the lesion area will be confirmed by a blinded ITRC).

These secondary analyses will be conducted on the PPS, FAS and ISS.

1.4.4 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, i.e. AESI.
- Occurrence of any Grade 3 or 4 AE possibly, probably or definitely related to 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 general AEs (body temperature, headache, myalgia, chills, nausea, fatigue, malaise, swollen lymph nodes) within 15 days after each vaccination (Days 0-14): Intensity, duration and relationship to vaccination.
- Occurrence of solicited local AEs (pain, redness (erythema), swelling, induration, itching (pruritus), and vaccination site appearance (normal/healed, red spot, bump, reddish blister

whitish blister, scab, ulcer/crater, warmth, swollen > 3inch, red streaks or drainage) within 15 days after each vaccination (Days 0-14): Intensity and duration.

- Daily measurement of lesion size, erythema and induration based on physical appearance of vaccination site as documented in the memory aid.

(i) major lesion diameter (mm) each day
(ii) major erythema diameter (mm) each day
(iii) major induration diameter (mm) each day

If the shape of the lesion, erythema (excludes lymphangitis) and induration observed is not round but rather asymmetrical then the largest (or major) cross-sectional measurement would be recorded.

The safety analyses will be conducted on the FAS.

1.5 Interim Analysis

No interim analysis is planned for this trial.

1.6 Data Safety Monitoring Board

The Data Safety Monitoring Committee (DSMB) is an independent expert panel appointed by the sponsor. This committee is in charge of surveying the subjects' safety throughout the course of the trial. All relevant roles, responsibilities and procedures for the DSMB are described in the DSMB charter. Since this is an open trial review will be done on unblinded summaries of the safety, disposition and demographic data prepared by the Chiltern biostatistician.

A summary of the activities and recommendations of the DSMB will be provided in the Clinical Study Report.

2 Trial Design

Table 1 Trial Procedure Schedule – Group 1

Visit (V)	SCR	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	Remote FU
Day / V +… d	-70- -1	0	V1 +6-8	V1 +13-15	V1 +27-29	V4 +6-8	V4 +13-15	V1 +55-57	V7 +6-8	V7 +13-15	V7 +27-29	Last Vaccination +182-210
Week	-10- -1	0	1	2	4	5	6	8	9	10	12	34-38
Procedures												
Informed consent	X											
Check inclusion / exclusion criteria	X	X										
Medical/Surgical History	X											
Check vaccination history and absence of vaccination scar	X											
Complete physical exam[1]	X											
Targeted physical exam[1]		X	(X)	X	X	(X)	X	X	(X)	X	X	(X)
Evaluation of vital signs	X	X	X	X	X	X	X	X	X	X	X	(X)
Recording of Baseline signs and symptoms	X	X										
Check cardiac risk factors	X											
ECG[2]	X			X			(X)			X		
Questions on cardiac signs and symptoms		X		X	X		X	X		X	X	
Review of prior/concomitant medications	X	X		X	X		X	X		X	X	(X)
Counseling[3]	X	X	X	X	X	X	X	X	X	X	X	
Examination of vaccination site		X	X	X	X	X	X	X	X	X	X	
Photo of vaccination site									X	X		
Bandage application / change[4]								X	X	X	X	
Assessment of axillary and supraclavicular lymphadenopathy		X	X	X	X	X	X	X	X	X	X	
SAE/AE/AESI recording		X	X	X	X	X	X	X	X	X	X	X[5]

Trial Procedure Schedule – Group 1 continued

Visit (V)	SCR	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	Remote FU
Day / V +… d	-70- -1	0	V1 +6-8	V1 +13-15	V1 +27-29	V4 +6-8	V4 +13-15	V1 +55-57	V7 +6-8	V7 +13-15	V7 +27-29	Last Vaccination +182-210
Week	-10- -1	0	1	2	4	5	6	8	9	10	12	34-38
Vaccination												
Randomization		X										
Vaccine administration		X			X			X				
Subject Observation (at least 30 minutes)		X			X			X				
Handout of memory aid[6]		X			X			X		X[7]		
Review of memory aid			X	X		X	X		X	X	X[7]	
Collection of memory aid[6]				X			X			X	X[7]	
Labs												
Pregnancy test[8]	X	X			X			X			X	
Safety lab[2] (CBC with differential, total bilirubin, Alk Phos, AST, ALT, creatinine, sodium, potassium and calcium)	X			X			X			X		(X)
Total, HDL and LDL cholesterol	X											
Hepatitis serology (HBsAg / Anti-HCV)	X											
HIV	X											
Troponin I testing[2]	X			X						X		
Blood draw for antibody analysis		X	X	X	X		X	X			X	
Blood volume												
Blood volume drawn per visit (ml)[9]	22	21	16	26	21		26	21		10	21	(10)
Cumulative blood volume drawn (ml)[9]	22	43	59	85	106		132	153		163	184	(194)

( ) Only indicated in case of medical need or any underlying condition that requires further examinations.

1 For more information on the physical examinations, please refer to Section 8.2.3 of the CTP.

2 Additional safety measures can be taken at any other trial visits or at unscheduled visits, if clinically indicated.

3 Counseling on avoidance of pregnancy and HIV infection and specifically after the ACAM2000® vaccination counseling on the avoidance of contact spread. At Visit 10 counseling for avoidance of pregnancy is not required.

4 After ACAM2000® administration subjects should change their own dressings as instructed by the administering clinic (leave the bandage on for the 1st 48 hours, then change every day or when bandage becomes wet) using the Care Kits provided by site staff until the vaccination site scab falls off, as this will keep the vaccination site intact and will minimize softening. Salves or ointments must not be applied to the vaccination site. Measurements of the vaccination site are to be taken until bandage not required.

5 Follow-up on ongoing AEs, AESIs, SAEs and recording of new SAEs/AESIs.

6 The memory aid should be completed daily for 15 days (day of vaccination and the following 14 days). If symptoms persist at 14 days post vaccination, temperature/symptom should be recorded until resolution or Visit 10.

7 If lesion at vaccination site has not healed at Day 14, measurements of lesion size, erythema and induration should be recorded on a memory aid extension each day until healed or Visit 10.

8 WOCBP only. At Screening Visit, a serum test must be performed. At all other visits, either a serum or urine pregnancy test will be performed.

9 Approximate volumes of single blood draws: Safety lab including all tests: 22 ml at SCR (including serum for pregnancy test) and 10 ml at regular visits,5 ml for serum pregnancy test and 16 ml for antibody analysis.

Table 2 Trial Procedure Schedule – Group 2

Visit (V)	SCR	V1	V2	V3	V4	V5	V6	Remote FU
Day / V +… d	-70- -1	0	V1 +6-8	V1 +13-15	V1 +27-29	V1 +41-43	V1 +55-57	Last Vaccination +182-210
Week	-10- -1	0	1	2	4	6	8	26-30
Procedures								
Informed consent	X							
Check inclusion / exclusion criteria	X	X						
Medical/Surgical History	X							
Check vaccination history and absence of vaccination scar	X							
Complete physical exam[1]	X							
Targeted physical exam[1]		X	(X)	X	X			(X)
Evaluation of vital signs	X	X	X	X	X			(X)
Recording of Baseline signs and symptoms	X	X						
Check cardiac risk factors	X							
ECG[2]	X			X				
Questions on cardiac signs and symptoms		X		X	X			
Review of prior/concomitant medications	X	X		X	X			(X)
Counseling[3]	X	X	X	X	X			
Examination of vaccination site		X	X	X	X			
Photo of vaccination site[4]			X	X				
Bandage application / change[4]		X	X	X	X			
Assessment of axillary and supraclavicular lymphadenopathy		X	X	X	X			
SAE/AE/AESI recording		X	X	X	X			X[5]

Trial Procedure Schedule – Group 2 continued

Visit (V)	SCR	V 1	V2	V3	V4	V5	V6	Remote FU
Day / V +… d	-70- -1	0	V1 +6-8	V1 +13-15	V1 +27-29	V1 +41-43	V1 +55-57	Last Vaccination +182-210
Week	-10- -1	0	1	2	4	6	8	26-30
Vaccination								
Randomization		X						
Vaccine administration		X						
Subject Observation (at least 30 minutes)		X						
Handout of memory aid[6]		X		X[7]				
Review of memory aid			X	X	X[7]			
Collection of memory aid[6]				X	X[7]			
Labs								
Pregnancy test[8]	X	X			X			
Safety lab[2] (CBC with differential, total bilirubin, Alk Phos, AST, ALT, creatinine, sodium, potassium and calcium)	X			X				(X)
Total, HDL and LDL cholesterol	X							
Hepatitis serology (HBsAg / Anti-HCV)	X							
HIV	X							
Troponin I testing[2]	X			X				
Blood draw for antibody analysis		X	X	X	X	X	X	
Blood volume								
Blood volume drawn per visit (ml)[9]	22	21	16	26	21	16	16	(10)
Cumulative blood volume drawn (ml)[9]	22	43	59	85	106	122	138	(148)

( ) Only indicated in case of medical need or any underlying condition that requires further examinations.

1 For more information on the physical examinations, please refer to Section 8.2.3 of the CTP.

2 Additional safety measures can be taken at any other trial visits or at unscheduled visits, if clinically indicated.

3 Counseling on avoidance of pregnancy and HIV infection and specifically after the ACAM2000® vaccination counseling on the avoidance of contact spread. At Visit 10 counseling for avoidance of pregnancy is not required.

4 After ACAM2000® administration subjects should change their own dressings as instructed by the administering clinic (leave the bandage on for the 1st 48 hours, then change every day or when bandage becomes wet) using the Care Kits provided by site staff until the vaccination site scab falls off, as this will keep the vaccination site intact and will minimize softening. Salves or ointments must not be applied to the vaccination site. Measurements of the vaccination site are to be taken until bandage not required.

5 Follow-up on ongoing AEs, AESIs, SAEs and recording of new SAEs/AESIs.

6 The memory aid should be completed daily for 15 days (day of vaccination and the following 14 days). If symptoms persist 14 days post vaccination, temperature/symptom should be recorded until resolution or Visit 4.

7 If lesion at vaccination site has not healed at Day 14, measurements of lesion size, erythema and induration should be recorded on a memory aid extension each day until healed or Visit 4.

8 WOCBP only. At Screening Visit, a serum test must be performed. At all other visits, either a serum or urine pregnancy test will be performed.

9 Approximate volumes of single blood draws: Safety lab including all tests: 22 ml at SCR (including serum for pregnancy test) and 10 ml at regular visits, 5 ml for serum pregnancy test and 16 ml for antibody analysis.

3 Statistical Methods and Considerations

3.1 Randomization Procedure

Target enrollment in the trial is 440 subjects to be vaccinated (220 subjects in Group 1 and 220 subjects in Group 2). Randomized treatment assignments for the subjects will be done after confirmation of subject's eligibility. Subjects will be allocated randomly into either Group 1 or Group 2 across the one clinical trial site in a 1:1 ratio. There will not be any stratification factors used in the randomization.

3.2 Immunogenicity Co-Primary Trial Hypothesis

Both co-primary trial hypotheses will be conducted on the PPS as the main analysis set. Similar analyses will also be conducted on the FAS and ISS.

The immunogenicity co-primary objective of the trial is to assess non-inferiority of MVA-BN® compared to ACAM2000® in terms of vaccinia-specific PRNT antibody response at the Peak Visits (Day 42 for Group 1 and Day 28 for Group 2).

It is assumed that the $\log_{10}$ titers for the PRNT are normally distributed with a mean of m1 in Group 1 and a mean of m2 in Group 2 and a common standard deviation (SD) for both groups.

The trial should demonstrate that the Day 42 GMT of Group 1 is not lower than the Day 28 GMT of Group 2 by more than a pre-specified amount. This amount is called the non-inferiority margin (Δ).

The test of non-inferiority will be applied for the following hypothesis:

H0: $m1 - m2 \leq -\Delta$ versus H1: $m1 - m2 > -\Delta$, where

Δ is the non-inferiority margin and is chosen in this trial as 0.301 on the $\log_{10}$ titers (i.e. a factor of ½ reported on the original titer scale).

Specifically, a standard homoscedastic one sided 97.5% lower confidence limit (LCL) of the difference between the log PRNT averages of the two groups will be calculated (Group 1 – Group 2). If the LCL is above –Δ then the null hypothesis will be rejected and the non-inferiority of MVA-BN® to ACAM2000® will have been demonstrated.

3.3 Efficacy Co-Primary Trial Hypothesis

3.3.1 The Hodges-Lehmann Estimate

We denote the set of observed MLAs for subjects $i = 1,2,\ldots,n_j$ in Group $j = 1$ (IMVAMUNE followed by ACAM2000 group) and Group $j = 2$ (ACAM2000 only group) as $x_{i,j}$. The observed sample median is denoted

$$\tilde{x}_j = \tfrac{1}{2}\left(x_{\lfloor (n_j+1)/2 \rfloor} + x_{\lceil (n_j+1)/2 \rceil}\right)$$

where $\lfloor (n_j+1)/2 \rfloor$ is the floor, or integer part of w rounded down, and $\lceil (n_j+1)/2 \rceil$ is the ceiling, or integer part rounded up. In the case of an even value of n_j then the floor and ceiling are the same value. The bracket notation $x_{(k)}$ denotes the k-th largest value in the set, or k-th order statistic, where $x_{(1)}$ and $x_{(n)}$ are the minimum and maximum values respectively.

Here we wish to calculate the confidence interval of the ratio of the medians, i.e. $r = \tilde{x}_1 / \tilde{x}_2$. The usual method of calculating the confidence interval of the ratio of the medians is to consider the logarithm of difference in the medians, i.e. $\log(r) = \log(\tilde{x}_1) - \log(\tilde{x}_2)$ and to calculate the confidence interval of the difference and then to take the antilogarithm to obtain the confidence interval for the ratio of medians. However, this presents problems due to possible zero values in the lesion area data. In fact, it is even possible that the median of Group 1 may be zero. To counteract this issue a small offset of 1 will be added to each zero value and the AREA Attenuation Rate (AAR) is calculated as:

$$AAR = 1 - \frac{\tilde{x}_1}{\tilde{x}_2} = 1 - r \qquad (1)$$

By subtracting the median ratio from 1 the AAR reflects the reduction in MLA.

Defining $y_{i,j} = \log(\max\{1, x_{i,j}\})$. It is assumed that $Y_{i,j}$ are independent identically distributed random variables from a continuous distribution, i.e.

$$\Pr(Y_{i,j} < u) = F_j(u) \qquad (2)$$

Similarly, we denote the MLDs (maximum lesion DIAMETERs) for subjects $i = 1,2,\ldots,n_j$ in Group $j = 1,2$ as $u_{i,j}$, with $v_{i,j} = \log(\max\{u_{i,j}1\})$, with zero MLD measurements replaced by 1 (as for the AAR calculations). The DIAMETER Attenuation Rate (DAR) is then:

$$DAR = 1 - \frac{\tilde{u}_1}{\tilde{u}_2}$$

Because no data has currently been confirmed for the MLA, it is not clear what the distribution of the MLA will be, and therefore it is sensible to use a non-parametric method.

For the HL method which will be used here, it is further assumed that both groups have the same distribution function but this has been shifted by an offset Δ for Group 2. This is referred to as the homogeneity of distribution assumption, i.e.

$$F_1(u) = F_2(u - \Delta) \qquad (3)$$

Next we define the set of $n_1 \times n_2$, so called, Wald differences as:

$$d_{ij} = \left(y_{i1} - y_{j2}\right) \quad \forall i, j$$

For current purposes an estimate of the shift difference is needed. The HL estimator (Hollander, 2014) is typically used to estimate the shift. Basically, the median of the Wald differences makes a very efficient estimator of the shift parameter, i.e.

$$\hat{\Delta} = \tilde{d} \tag{4}$$

The Mann-Whitney test statistics, denoted U, is defined as the number d values below zero. The U statistic which has the following expectation and variance under the assumption that $\Delta=0$:

$$E[U] = \tfrac{1}{2} n_1 n_2$$

$$V[U] = \frac{n_1 n_2 (n_1 + n_2 + 1)}{12}$$

One advantage of the HL estimate is that it has a straightforward way to estimate a 100(1-α)% confidence interval denoted $\left(\hat{\Delta}_L, \hat{\Delta}_U\right)$ attributed to Lincoln Moses (Hollander, 2014).

We specify the order statistics of the set of Wald differences as $\left\{d_{(1)}, d_{(2)}, \ldots, d_{(n_1 n_2)}\right\}$, then based on a large sample size approximation to the normal distribution we find the 95% lower and upper confidence limit of U, called k_L and k_U respectively, as

$$k_L = \left\lceil E[U] - 1.96\sqrt{V[U]} \right\rceil \text{ and } k_U = \left\lceil E[U] + 1.96\sqrt{V[U]} \right\rceil$$

Since this is a discrete distribution, the point k is actually the integer that just exceeds the upper confidence limit of U. Then we match the corresponding values of $\left(d_{(k_L)}, d_{(k_U)}\right)$ as the estimate of the confidence interval.

The exact distribution of U (and hence k) can be found, but this can be computationally intense for large sample sizes, and the above normal approximation is then very accurate.

In general the HL estimator is estimating the shift Δ (although under the homogeneity of distribution assumption this should be the same as the difference in the two medians). The HL estimator has excellent efficiency and is at least 86.6% (Hollander, 2014) as efficient as any other estimator of the shift parameter. Even in the case that the data are normally distributed, which would be a very efficient parametric model, the HL estimator is 95.5% as efficient as the student t-test. However, when the data is not normally distributed it can be much more efficient than the mean difference estimator.

The HL estimator is also much more efficient than the median difference estimator which is rather insensitive (Høyland, 1965). The HL estimator is usually used, as here, as an estimator of the difference in the group medians. However, for comparison the HL estimator of the shift will also be presented. In addition, a secondary supportive analysis based on the PB method, which directly estimates the difference in the medians will also presented (see below).

3.3.2 Efficacy Hypothesis

The efficacy co-primary hypothesis of the trial is to assess if the median MLA in Group 1 is reduced compared to that in Group 2. This is done by considering the AAR defined in Equation (1), and whether this is significantly greater than or equal to a pre-specified factor λ=0.4:

$$H_0: \ AAR \leq \lambda \qquad \text{versus} \qquad H_1: \ AAR > \lambda \tag{5}$$

This is tested using the anti-log of the 95% HL confidence interval (Lehmann, 1975) defined in Equation (4). Specifically, only the upper 95% confidence interval of the HL shift estimator is used with a one-sided significance level of 2.5%, and this needs to be below (1-λ)=0.6 for the null hypothesis to be rejected, and thus the co-primary endpoint will be met. Otherwise, the null hypothesis will not be rejected and the co-primary endpoint will not have been met. However, for completeness the anti-logs of the upper and lower confidence limits along with the point estimate of the shift estimator, and ratio of the medians will be reported.

3.3.3 Justification of λ

The value of λ is defined to be 40%, in other words so that a significant relative reduction of MLA of at least 40% in Group 1 compared to Group 2 is shown. The 40% reduction is considered a clinically significant difference as this is the level of reduction that has been seen in subjects previously vaccinated with Dryvax® and then revaccinated with a first generation smallpox vaccine (Wetvax) (Talbot, 2006). In this trial, 48 vaccinia-naïve subjects and 54 vaccinia-experienced subjects were vaccinated with Wetvax and treated with one of three different bandages (Gauze, Occlusive or Foam). The focus of the trial was to determine if the choice of bandage had an effect on the development of the subsequent take with a view to reducing possible re-infection rates. One of the take, or lesion, parameters measured was the maximum lesion area measured during the 14 days post scarification. The use of naïve and experienced subjects allows the comparison of the maximum lesion diameter obtained for naïve and experienced subjects. Since first generation smallpox vaccines are considered to give lifelong protection the differences observed in lesion diameters can be used as a measure of the level of attenuation that will be obtained for protected (i.e. experienced subjects). In the Talbot trial the Gauze and Occlusive bandages gave relative reductions in the median lesion diameter that equate to a relative reduction of experienced to naïve subjects lesion area of 41.5% and 37.7% respectively (assuming lesions are circular). The foam bandage gave a relative reduction of only 11.4%. The foam bandage is very different to the bandage that will be used in this trial, and hence taking a value of 40% for λ is considered a good estimate of clinical efficacy.

3.3.4 Numerical Example

The following is an illustration of the above methods using a real numerical example. The best source of information for the distribution of the MLD data available using IMVAMUNE is from the NIH sponsored POX-MVA-002 trial (Frey, 2007). In the POX-MVA-002 trial some subjects were given either Placebo/Placebo/Dryvax® (P/P/D), which is Group T1 in the trial; or MVA-BN®/MVA-BN®/Dryvax® (M/M/D), which is called group T2 in the trial. After the Dryvax® vaccination, the maximum diameter of the lesion was measured at various visits, including the Day 6-8 Visit and the Day 13-15 Visit. The raw data is given in Table 3 below

Table 3 Lesion Data from POX-MVA-002 clinical trial

Treatment Group = MVA/MVA/Dryvax® (M/M/D) N =12				
Subject ID	MLD (mm)	$\log(MLD)$	MLA* (mm²)	$\log(MLA)$
[redacted]	0	0	0	0
[redacted]	0	0	0	0
[redacted]	2	0.301	4	0.602
[redacted]	4	0.602	16	1.204
[redacted]	5	0.699	25	1.398
[redacted]	7	0.845	49	1.690
[redacted]	7	0.845	49	1.690
[redacted]	7	0.845	49	1.690
[redacted]	7	0.845	49	1.690
[redacted]	8	0.903	64	1.806
[redacted]	9	0.954	81	1.908
[redacted]	10	1.000	100	2.000
Median	7	0.845	49	1.690

Treatment Group = Placebo/Placebo/Dryvax® (P/P/D) N =13				
Subject ID	MLD (mm)	$\log(MLD)$	MLA* (mm²)	$\log(MLA)$
[redacted]	9	0.954	81	1.908

[redacted]	10	1.000	100	2.000
[redacted]	10	1.000	100	2.000
[redacted]	10	1.000	100	2.000
[redacted]	10	1.000	100	2.000
[redacted]	11	1.041	121	2.083
[redacted]	12	1.079	144	2.158
[redacted]	13	1.114	169	2.228
[redacted]	13	1.114	169	2.228
[redacted]	15	1.176	225	2.352
[redacted]	17	1.230	289	2.461
[redacted]	20	1.301	400	2.602
[redacted]	20	1.301	400	2.602
Median	12	1.079	144	2.158

*MLA is imputed using the rounded value of MLD^2

The MLA was imputed by assuming it was the square of the MLD, strictly speaking if we assume the lesion is exactly circular then the area is ¼π of the MLD squared. However, the factor ¼π will cancel out when we consider the ratio of the medians, and so for simplicity we just use the square of the MLD.

A histogram of the MLD data is given in Figure 1 below. In the figure it is clear that the data do not remotely follow a Normal distribution, as they are positive and highly skewed. Therefore a student t-test of the difference between the groups is not appropriate, and it seems prudent to adopt a non-parametric approach for calculating the AAR.

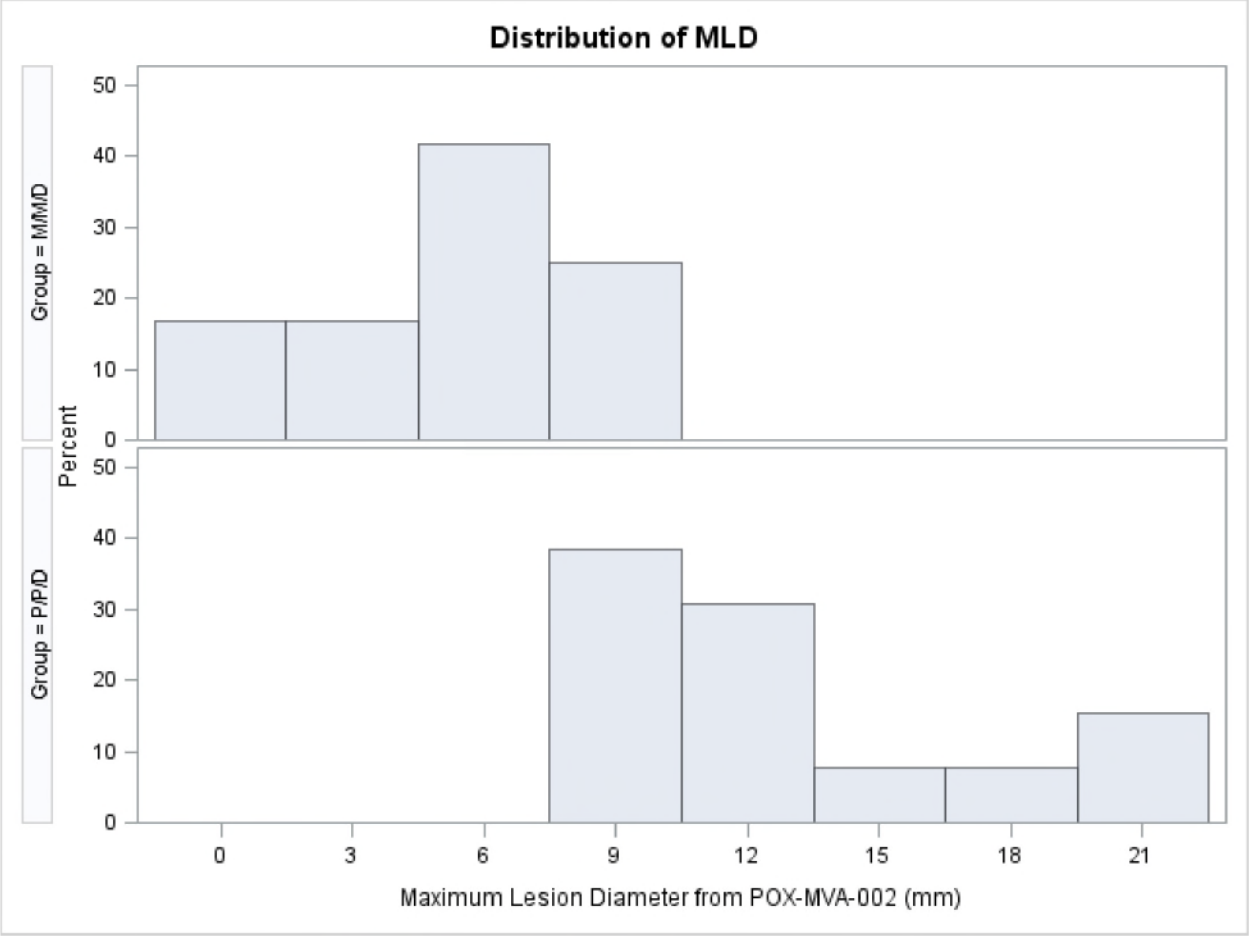

Figure 1 Histogram of MLD from POX-MVA-002

The ranked Wald differences ($d_{(i)}$) of the log MLD values generate the following set of 12×13 = 156 values given in Table 4 below. From Table 3 above the DAR = 1 - 7/12 = 0.417. The shift estimator of the log MLA is $\hat{\Delta} = \frac{1}{2} d_{(78)} + \frac{1}{2} d_{(79)} = -0.331$ and taking 1 minus the anti-log the asymptotic HL estimate is 0.533. This is a little different from the observed DAR, but the sample size is not large in this example.

Then $E[U] = 78$ and $V[U] = 338$, hence $k = \lceil 78 + 36.03 \rceil = 115$ and $d_{(115)} = -0.155$ with $\lfloor 78 - 36.03 \rfloor = 42$ and $d_{(42)} = -0.628$, with the 95% HL CI of the DAR being (0.300, 0.765). Since the lower limit of the confidence interval is below 0.4 this would not meet the hypothesis as specified above. This is not surprising since these are lesion diameters not areas and the sample size is small.

Table 4 Wald differences of log MLD values from POX-MVA-002 clinical trial

(i)	$d_{(i)}$	(i)	$d_{(i)}$	(i)	$d_{(i)}$	(i)	$d_{(i)}$	(i)	$d_{(i)}$	(i)	$d_{(i)}$
1	-1.301	27	-0.954	53	-0.456	79	-0.331	105	-0.211	131	-0.138
2	-1.301	28	-0.954	54	-0.456	80	-0.331	106	-0.211	132	-0.125
3	-1.301	29	-0.929	55	-0.456	81	-0.327	107	-0.196	133	-0.114
4	-1.301	30	-0.875	56	-0.456	82	-0.301	108	-0.196	134	-0.114
5	-1.230	31	-0.813	57	-0.456	83	-0.301	109	-0.196	135	-0.109
6	-1.230	32	-0.813	58	-0.456	84	-0.301	110	-0.196	136	-0.109
7	-1.176	33	-0.778	59	-0.439	85	-0.301	111	-0.176	137	-0.109
8	-1.176	34	-0.740	60	-0.415	86	-0.301	112	-0.176	138	-0.109
9	-1.114	35	-0.699	61	-0.415	87	-0.301	113	-0.160	139	-0.097
10	-1.114	36	-0.699	62	-0.398	88	-0.276	114	-0.160	140	-0.097
11	-1.114	37	-0.699	63	-0.398	89	-0.273	115	-0.155	141	-0.097
12	-1.114	38	-0.699	64	-0.398	90	-0.269	116	-0.155	142	-0.097
13	-1.079	39	-0.699	65	-0.398	91	-0.269	117	-0.155	143	-0.087
14	-1.079	40	-0.699	66	-0.398	92	-0.269	118	-0.155	144	-0.079
15	-1.041	41	-0.653	67	-0.398	93	-0.269	119	-0.155	145	-0.051
16	-1.041	42	-0.628	68	-0.385	94	-0.269	120	-0.155	146	-0.046
17	-1.000	43	-0.602	69	-0.385	95	-0.269	121	-0.155	147	-0.046
18	-1.000	44	-0.602	70	-0.385	96	-0.269	122	-0.155	148	-0.046
19	-1.000	45	-0.574	71	-0.385	97	-0.269	123	-0.155	149	-0.046
20	-1.000	46	-0.531	72	-0.380	98	-0.255	124	-0.155	150	-0.041
21	-1.000	47	-0.512	73	-0.352	99	-0.234	125	-0.155	151	0.000
22	-1.000	48	-0.512	74	-0.347	100	-0.234	126	-0.155	152	0.000
23	-1.000	49	-0.477	75	-0.347	101	-0.234	127	-0.155	153	0.000
24	-1.000	50	-0.477	76	-0.342	102	-0.234	128	-0.155	154	0.000
25	-1.000	51	-0.456	77	-0.331	103	-0.230	129	-0.155	155	0.000
26	-1.000	52	-0.456	**78**	**-0.331**	104	-0.222	130	-0.155	156	0.046

For the imputed MLA the median in Group T2 (IMVAMUNE then Dryvax) is 49 and the median in Group T1 (Dryvax only) is 144, and the imputed AAR = 1 – 49/144 = 0.660.

The difference in the log10 medians is then 1.690 – 2.158 = -0.468, with 95% HL CI of (-1.204, -0.320). So, taking 1 minus the anti-logs the 95% CI of the AAR is (0.521, 0.937). Because the lower limit is above 0.4 the primary endpoint would have been achieved using this example.

3.4 Secondary Immunogenicity Hypotheses

The most important secondary immunogenicity objective of the trial is to assess non-inferiority of MVA-BN® compared to a conventional smallpox vaccine (ACAM2000®) in terms of antibody response at the Peak Visit, i.e. 28 days after an ACAM2000® vaccination and 42 days after the first MVA-BN® vaccination using the vaccinia-specific ELISA.

The test of non-inferiority will be applied in the same way as described above for the PRNT GMTs except it will be carried out for the ELISA GMTs, i.e. the following hypothesis will be tested:

$$H_0: m1 - m2 \leq -\Delta \text{ versus } H_1: m1 - m2 > -\Delta,$$

where Δ is the non-inferiority margin and is chosen in this trial as 0.176 on the $\log_{10}$ titers (i.e. a factor of ⅔ reported on the original titer scale).

In addition, two further secondary non-inferiority analyses of ELISA and PRNT titers will be conducted for the individual peak titers, i.e. for each subject in Group 1 the individual maximum titer from Visit 1 to Visit 7 will be used, and in Group 2 the individual maximum titer from Visit 1 to Visit 6 will be used. The analyses will proceed in the same way as outlined above for the PRNT and ELISA non-inferiority analyses including the use of the same respective non-inferiority margins.

3.5 Secondary Efficacy Hypotheses

We might be concerned whether the homogeneity of variance assumption for the HL method is correct. In one way this is checked by presenting both the observed ratio of the medians and the HL shift estimator. However, as a secondary supportive analysis, a direct estimation of the confidence interval of the medians is also performed using the PB method (see Price and Bonett, 2002). Here the (1-α) confidence interval for the ratio of medians (r) is defined as

$$r \exp\left\{\pm z_{\alpha/2}\left[\mathrm{var}(\tilde{y}_1) + \mathrm{var}(\tilde{y}_2)\right]^{1/2}\right\} \qquad (4)$$

Where $\mathrm{var}(\tilde{y}_j)$ is an estimate of the variances of $\tilde{y}_j$ and $z_{\alpha/2}$ is the usual standard normal score, i.e. for a 95% confidence interval it is the value 1.96. The PB estimates of the variances are then

$$\mathrm{var}(\tilde{y}_j) = \left\{\frac{\left(y_{(n_j - c_j + 1),j} - y_{(c_j),j}\right)}{2z_j}\right\}^2$$

With $c_j = \frac{1}{2}(n_j + 1) - \sqrt{n_j}$ rounded to the nearest integer and $z_j = \Phi^{-1}\left(1 - \frac{1}{2}p_j\right)$, and $p_j = \sum_{i=1}^{c_j - 1} \frac{n_j!}{(n_j - i)!i!}(0.5)^{n_j - 1}$.

The PB CI will be calculated alongside all examples where the HL interval is calculated.

For the POX-MVA-002 example the median MLD in Group *T*2 (IMVAMUNE then Dryvax) is 7 and the median in Group *T*1 (Dryvax only) is 12, and hence the observed DAR = 0.417 with 95% PB CI of (-0.233, 0.692), the lower limit of which is negative. As noted previously, the PB method is considerably less powerful than the HL method.

Historically, the MLD has been used as a basis for measuring take attenuation. The MLA (maximum lesion AREA) is used in this trial as co-primary endpoint because it will present a more accurate measurement than using the diameter. That said, for comparison and historical purposes it is of interest to consider the MLD as a secondary supportive analysis.

The HL 95% confidence interval (and PB) will also be calculated for the DAR using the same method as defined in (4).

Secondary analyses will also be performed on separately for lesion diameter measurements and lesion area measurements on Day 6-8 and on Day 13-15.

3.6 Sample Size

The current sample size for this trial represents something close to the maximum number of subjects that can be recruited within the trial duration and should provide sufficient power for the co-primary endpoints to meet their respective objectives.

This means that the overall power for both co-primary endpoints meeting the objectives is at least 80%, i.e. should be at least 90% for each co-primary endpoint (i.e. 90% × 90% > 80%).

Since it is required to meet both co-primary objectives, the overall sample size is fixed as the larger sample size required for each co-primary endpoint to be met with 90% power.

3.6.1 Immunogenicity Co-Primary Endpoint

The immunogenicity co-primary objective of the trial is to demonstrate the non-inferiority of MVA-BN® to a conventional smallpox vaccine (ACAM2000®) in terms of PRNT antibody response (GMTs) at the Peak Visit. Hence the following sample size calculation was performed for the co-primary immunogenicity endpoint.

Assuming a standard significance level alpha of 5%, a power of ≥ 90% and the same expected individual peak PRNT GMTs in the MVA-BN® group (Group 1) and the ACAM2000® group (Group 2), then the only remaining parameter is the SD of the $\log_{10}$ titers.

As a basis for calculation, the combined calculated value of the SD at Day 42 of the $\log_{10}$ titers for healthy vaccinia-naïve subjects who received the standard MVA-BN® vaccination schedule was taken from three Phase II MVA-BN® studies (POX-MVA-005, POX-MVA-008 and POX-MVA-011). In total this was 419 healthy subjects and the calculated SD is 0.807 with a 95% confidence interval of (0.756, 0.866).

Adopting a worst case scenario and using the upper limit of the confidence interval, i.e. assuming SD = 0.866, this yields a required sample size for this trial of 175 in the PPS in both groups.

In order to account for an estimated 20% rate for exclusion from the PPS (verbal communication from USAMRIID as experience from their trials in this population), a total of 220 subjects will be recruited into each of the groups.

3.6.2 Efficacy Co-Primary Endpoint

The efficacy co-primary objective is to determine if take attenuation has occurred in Group 1 compared to Group 2 as specified in Section 3.3.2.

To calculate the likely power for this analysis data from the POX-MVA-002 trial was used as a basis for calculations. Using this data a simple case resampling non-parametric bootstrap simulation was performed to estimate the likely AAR. Specifically, a random sample of size n was drawn with replacement from each group. Each individual MLD was then adjusted by a uniform random variable on the range [-½, ½]. The MLA was imputed by assuming it is the MLD (worst case). Then, 10,000 bootstrap samples were generated. For each bootstrap sample the observed AAR was calculated. The 10th percentile of the bootstrapped lower confidence limits of the median of the differences then gives an estimate of the value of lambda that can be achieved with 90% power for a given group sample size, see the Table 5 below (also for 95% power and 99% power).

Table 5 Sample size calculations for the Efficacy Endpoint

n (per group) in PPS	60	75	90	105	120	150	175
Power for λ=0.4	87.0	92.8	96.3	98.0	99.1	99.8	99.9
λ that achieves 90% Power	39.1	41.0	42.2	43.0	43.7	44.8	45.3
λ that achieves 95% Power	37.2	39.2	40.6	41.7	42.5	43.8	44.4
λ that achieves 99% Power	34.3	36.1	37.4	38.9	40.1	41.8	42.7

From the above simulation results the power of passing the co-primary endpoint based on the AAR is above 90% for sample sizes of at least n=75 in the PPS. However, since it is known that bootstrap simulations can be somewhat optimistic and the POX-MVA-002 trial is small and has differences to those in the current POX-MVA-006 trial, it therefore seems sensible to choose a sample size at least somewhat bigger than the minimum n=75. Regardless, the proposed sample size of n=175 per group required for the immunogenicity co-primary endpoint should easily be adequate for the efficacy endpoint.

3.7 Analysis Populations

The following analysis data sets will be defined for the statistical analyses.

3.7.1 Full Analysis Set

The Full Analysis Set (FAS) consists of all subjects

i) who had received at least one dose of trial vaccine and

ii) for whom any post vaccination safety or immunogenicity data are available

The safety analysis and secondary supportive immunogenicity analyses will be performed on the FAS. Subjects in Group 1 who do not receive an MVA-BN® vaccination will not be considered enrolled in the trial (although they should receive an ACAM2000® vaccination outside of the protocol later).

3.7.2 Per Protocol Set

The Per Protocol Set (PPS) is the subset of subjects in the FAS who have received all vaccinations, completed all visits up until Visit 10 for Group 1 and Visit 4 for Group 2, and adhered to all protocol conditions. Subjects with only minor (not relevant) protocol deviations will be included into this dataset.

The decision whether a protocol deviation is major or minor will be made on a case-by-case basis in a data review meeting (DRM) prior to database lock.

Examples of major protocol violation are:

1. Premature discontinuation of the trial (the question "prematurely terminated the trial?" is answered with "yes" even where no other reason exists to exclude the subject from further participation in accordance with the protocol)
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 fulfils 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 DRM
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 primary endpoint dataset will be the PPS. All confirmatory testing is based on this subgroup. For further descriptive purposes, the same statistical procedures will be applied to the FAS.

3.7.3 Initially Seronegative Subset

As seen in supposedly vaccinia-naive populations in previous MVA-BN® trials, some subjects will most likely be initially tested seropositive at Visit 1 prior to vaccination in either the ELISA or PRNT. An additional subgroup analysis based on the subset of the PPS of initially seronegative subjects, the Initially Seronegative Subset (ISS) will therefore be performed which will provide assurances that the results have not been affected by the possible inclusion of some vaccinia-experienced subjects. In doing so it should be noted that some of the initially seropositive values might be false positive values. However, there is no reliable way to determine which seropositive cases are false positive and which are true positive values, and the ISS is therefore a conservative approach to validating the results in a confirmed naïve population.

A subject of the PPS will be part of the ISS, if the subject is seronegative for both ELISA and PRNT at baseline.

The ISS population will be used as an additional robustness analysis set for the immunogenicity and the demographic analyses.

3.8 Definitions, Data Conventions and Handling of Missing Data

3.8.1 Missing Data

Analysis of immunogenicity variables will be done on a valid case basis, i.e. for missing observations no imputation technique such as "Last observation carried forward" (LOCF) will be 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 will be imputed in order to assign these events to the correct vaccination period (see Section 3.8.2).

Missing Vital Signs data for Visit 1 will be imputed using screening (or latest re-screening) values. However, this will be displayed as 'Baseline' values in the tables and listing for Vital Signs data without reference to whether the data originated from Visit 1 or SCR.

All data will be listed and summarized as captured in the eCRF. However, it may be necessary to impute incomplete AE and medication start and end dates in order to assign these events to the correct vaccination period, which will be done as follows:

For prior and concomitant medication and AEs imputation of partial start and end dates will be done for analysis purpose according to the following rules:

Missing	Rule for start date	Rule for end date	Flag for imputation
Day	First of month*	Last of month*	D
Month†	1. January*	31. December*	M
Year†	no imputation	Last visit date	Y

* Unless the imputed start date is before first visit date, in which case first visit date is used; or the imputed stop date is after last visit date in which case last visit date available is used.

† It is assumed that a missing month implies a missing day as well, and that a missing year implies a missing month and day.

All listings will display the original dates as captured in the eCRF.

For analysis of efficacy data if one or both of the Day 6-8 or Day 13-15 photographs are missing then the photograph will be recorded as missing and the assessment of attenuation will not be made.

3.8.2 Assignment of AEs to Vaccination Period

Each AE will be assigned to a vaccination period using date and time of vaccination and date and time of start of AE. If not specified otherwise all AE tables will be presented divided into the following vaccination periods:

For Group 1:

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

For Group 2:

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

If start time is missing and start date of AE coincides with the date of a vaccination, the AE will be assigned to the vaccination period corresponding to the vaccination on this date.

Each AE starting at or after first vaccination not matching the definition of a solicited AE is defined as an unsolicited AE. If a solicited AE begins outside of the 15-day window following the last vaccination it will be considered an unsolicited AE regardless of the preferred term. If start time is missing and start date coincides with date of current vaccination, it will be regarded as a treatment emergent AE. If the start date is (partially) missing the AE will be regarded as a treatment emergent AE following the worst case principle. If the AE cannot be assigned to a vaccination period 1 or 2 because of a (partially) missing start date it will be assigned to the overall MVA-BN® vaccination period (Group 1). If the AE cannot be assigned to vaccination period 2 or 3 then it will not be assigned to a vaccination period. AEs which cannot be assigned

to a vaccination period will still be included in per subject based tables and in the listings (with partial dates).

AEs with onset after the active trial phase will be considered to fall within the FU phase.

3.8.3 General Considerations for AEs

MedDRA (Medical Dictionary for Regulatory Activities) version 18.0 will be used for coding of AEs.

Duration of an AE is calculated as follows:

Unsolicited AE: expressed in [days]

- end date of AE – start date of AE + 1
- in case of (partially) missing start date and/or (partially) missing end date imputed dates will be used in the calculation.
- in case the AE is ongoing at the end date, the duration will not be calculated

Solicited AEs: expressed in [days]

- end date of AE – start date of AE + 1 where end date is the last day the symptom is defined as an AE and start date is the first day symptom is defined as an AE (no matter if the AE occurred at every day between first day and last day).
- in case the AE is ongoing at the end date, the duration will not be calculated

Time interval between vaccination and start of AE are calculated as follows:

Relative day of start of AE is calculated as follows:

Unsolicited AEs: expressed in [days]

- start date of AE – date of vaccination of the corresponding period
- in case of (partially) missing start date no calculation will be done.

Solicited AEs: expressed in [days]

- start date of AE – date of vaccination of the corresponding period where start date is the first day a symptom is defined as an AE, and vaccination day corresponds to the day of vaccination for each period.

Local and General Unsolicited AEs

Any unsolicited AE which is recorded with the preferred term including the text “injection site” or “vaccination site” will be considered as a local reaction. Any other preferred terms which are

considered as local events will be documented in the DRM minutes. Any unsolicited AE not classified as a local AE will be classified as a General AE.

All available safety data collected during the FU phases will therefore be simply listed. However the new onset SAEs reported during the FU phase will be analyzed.

3.9 Analysis Variables

3.9.1 Demographic and Other Baseline Characteristics

Demographics

- Age (years)
- Age bands (15-24 years, 25-34 years, 35-44 years)
- Gender
- Race (American Indian or Alaskan Native, Oriental/Asian, Black/ African American, Native Hawaiian / Other Pacific Islander, White/Caucasian, Other)
- Ethnicity (Hispanic or Latino, or Non-Hispanic or Latino)
- Height [cm]
- Body weight [kg]
- Body Mass Index (BMI)

Other Baseline characteristics

- History of smallpox vaccination or vaccination with other pox-virus based vaccine
- Medical history (including cardiac risk assessment)
- Baseline signs and symptoms pre-existing to vaccination

3.9.2 Safety Variables

Physical examination (complete examinations at Visit SCR and targeted physical examination at other visits).

Only abnormal physical examination results will be listed.

Vital signs (at each visit)

- Heart rate [beats per minute]
- Systolic and diastolic blood pressure [mmHg]
- Body temperature [°F]
- Respiratory rate (breaths/min)

12-lead electrocardiogram (ECG) (at SCR and Visit 3 and in Group 1 only Visit 9)

(Additional ECGs will be performed and reported if clinically indicated [e.g. in Group 1 at Visit 6], or in case of any clinically significant cardiac events are present)

- Investigator's overall interpretation (normal, abnormal)
- Clinical significance

Safety laboratory data (at SCR, Visit 3 and in Group 1 only Visit 6 and Visit 9)

Clinical chemistry (serum)

- Total bilirubin
- Alkaline phosphatase
- SGOT/AST (Aspartate Aminotransferase)
- SGPT/ALT (Alanine Aminotransferase)
- Serum creatinine
- Sodium
- Potassium
- Calcium
- Troponin I (at SCR and Visit 3 and in Group 1 only Visit 9)

And the following only at SCR

- LDL
- HDL
- Hepatitis B/C –Testing
- HIV Testing
- Total cholesterol

Hematology (whole blood)

- Red blood cell count
- Hemoglobin
- Total and differential WBC (Eosinophils, Basophils, Neutrophils - % and absolute -, Leucocytes, Lymphocytes, Monocytes)
- Platelet count

Pregnancy test (serum β-HCG pregnancy test at SCR or urine β-HCG pregnancy test within 24 hours prior to each vaccination and at last active trial visit for women of childbearing potential.

Results of all pregnancy tests will be listed.

Solicited local AEs reported in the subject memory aid (on days of vaccination and during the following 14 days)

- Injection Site Erythema (Redness)
- Injection Site Swelling
- Injection Site Pain
- Injection Site Induration
- Injection Site Itching (Pruritus)

And vaccination site appearance (normal/healed, red spot, bump, reddish blister, whitish blister, scab, ulcer/crater, warmth, swollen >3inch, red streaks, drainage)

Solicited general AEs reported in the subject memory aid (on days of vaccination and during the following 14 days)

- Body Temperature (Fever)
- Headache
- Myalgia (Muscle pain)
- Chills
- Nausea
- Fatigue
- Malaise
- Swollen Lymph nodes (Lymphadenopathy)

Unsolicited AEs reported (on days of vaccination and during the following 28 days)

- Other, unsolicited local and general adverse events following vaccination

SAEs

AESI

In case any AESI is also an SAE it will be treated and reported as an SAE.

Prior and Concomitant medication

Assessment of ACAM2000® Vaccination Site

The following four measures will be derived from the subject's memory aids:

- healing time measured in days

- maximum lesion diameter measured in mm
- maximum erythema measured in mm
- maximum induration measured in mm

The following measurements will be made by the investigator at Day 0, Day 7, Day 14 and Day 28 visits following ACAM2000® vaccination

- lesion erythema measured in mm
- lesion induration measured in mm
- general appearance of the lesion

3.9.3 Immunogenicity and Efficacy Variables

Seropositivity (yes/no) at all sampling visits

- Vaccinia-specific neutralizing antibodies (PRNT)
- Total vaccinia-specific antibodies (ELISA)

Seroconversion (yes/no) at all post baseline sampling visits

- Vaccinia-specific neutralizing antibodies (PRNT)
- Total vaccinia-specific antibodies (ELISA)

Antibody titers at all sampling visits

- Vaccinia-specific neutralizing antibodies (PRNT)
- Total vaccinia-specific antibodies (ELISA)

The following assessments will be made using the SilhouetteConnect camera system (using the area perimeter drawn on the photograph)

- Lesion area at Day 6-8
- Lesion area at Day 13-15
- Lesion diameter at Day 6-8
- Lesion diameter at Day 13-15

The lesion area measurements will be confirmed by the blinded ITRC. Where there is a discrepancy, then the ITRC would have the final decision.

The following assessments will be made by the investigator

- Lesion diameter at Day 6-8
- Lesion diameter at Day 13-15
- Healing of lesion assessed at visits on Day 7, Day 14 and Day 28.

- Major erythema at Day 6-8
- Major erythema at Day 13-15
- Major induration at Day 6-8
- Major induration at Day 13-15

The following assessment will be made by the blinded ITRC:

- ITRC classification of lesion site (full take, partial take, absent take)
- MLA

3.9.4 Pharmacokinetic Variables

Not applicable

3.9.5 Pharmacodynamic Variables

Not applicable

3.10 Analysis and Presentation Methods

3.10.1 Listings and Descriptive Statistics

All individual data entered in the eCRF and derived data will be listed as measured in the Individual Subject Data Listing.

For ELISA and PRNT titers descriptive statistics will be based on Geometric Means and confidence intervals (CI). For other, continuous parameters, measurements will be summarized by means of descriptive statistics (i.e. number of observations (N), mean, SD, minimum (Min), median, maximum (Max)) and categorical data will be summarized by means of frequency tables (i.e. count and percentages) and CI, if not stated otherwise.

All tables and listings will be sorted by scheduled visit (and subject, if appropriate).

All tables will be presented split by treatment groups (Groups 1 and 2). A comparison of the ACAM2000® vaccination phase will also be presented between Groups 1 and 2.

Repeat assessments/measurements and unscheduled assessments/measurements will be included in the Individual Subject Data Listing. All other repeat values will be listed in a separate listing.

3.10.2 Software

All statistical summaries and analyses of safety and efficacy data will be performed using SAS® 9.2 or higher (Statistical Analysis System, SAS-Institute, Cary, NC, USA) for Windows XP.

3.10.3 Disposition of Subjects

All subjects screened will be accounted for. A summary table will be presented specifying:

- The number of subjects screened
- The number of subjects randomized
- The number of subjects completing the active phase of the trial
- The number of subjects included in each analysis set
- The number of subjects withdrawn from the second vaccination (Group 1 only)
- The number of subjects prematurely discontinuing the trial (as well as the frequency of primary reasons for withdrawal/premature discontinuation)
- The number of subjects withdrawn from the third vaccination (Group 1 only)
- The number of subjects participating in remote (phone or email) FU Visit
- The number of subjects attending an on-site FU Visit

A listing will present all randomized subjects, time and date of completion or discontinuation with date of last dose of trial medication, date of discontinuation, reason for discontinuation and primary reason for withdrawal from second or third vaccination, if applicable.

All screening failures (subjects not eligible for the trial) will be listed including the reason why not eligible. Listings will also be presented for all violations in inclusion criteria and all exclusion criteria fulfilled.

All listings will be based on the screened subjects with screen failures and subjects randomized but not treated considered as two separate trial groups. However tables of the descriptive statistics for the demographics will only be produced for the FAS and PPS.

3.10.4 Demographic and Other Baseline Data

Descriptive statistics will be presented for the continuous demographic variables, age, age band, height, and body weight. Note that the date of the actual Screening Visit is used for age, age band, height and body weight calculations (hence also for BMI) and not the date of any Re-screening Visit.

Age is calculated using the following formula:

AGE = Year of Screening Visit – Year of Birth;
if Month of Screening Visit < Month of Birth then AGE → AGE – 1;
if Month of Screening Visit = Month of Birth
and Day of Screening Visit < Day of Birth then AGE → AGE – 1

Frequencies and percentages of subjects will be tabulated for the categorical variables sex, race and ethnicity in the same table by treatment group and overall. Percentages will be based on the total number of subjects in the relevant population.

Descriptive statistics for the demographic data will be produced by treatment group.

A Wilcoxon test for differences of the main baseline characteristics (age, body weight, height, BMI) will be performed between treatment groups.

A Fisher's exact test will be used to compare the gender, race and ethnicity between both treatment groups.

A summary of medical history by Preferred Term (PT) and System Organ Class (SOC) will be produced for each group.

Baseline signs and symptoms will be listed by body system and preferred term. Relative day of onset and duration of Baseline signs and symptoms will be calculated as described in Section 3.8.3. Baseline signs and symptoms are defined as AEs with start date after the subject was screened for the trial but before the first vaccination was received. These events are to be reported.

3.10.5 Prior and Concomitant Medication

All prior medication will be summarized by Anatomical-Therapeutical-Chemical (ATC) class and Generic name according to the 2015/Q1 version of the World Health Organization Drug Dictionary (WHO-DD). All concomitant medication will be summarized by ATC class and Generic name for all subjects in the FAS.

The table/listing "Prior medication" includes the medication data where end date is before date of first administration of trial vaccine. The table/listing "Concomitant medication" includes ongoing medication or medication with missing end date or with end date after date of first administration of trial vaccine.

All listings will display the original dates as captured in the eCRF.

3.10.6 Compliance

Compliance will be evaluated with respect to the number of vaccine doses received and the number of memory aids completed.

3.10.7 Immunogenicity Analysis

The co-primary immunogenicity objective of the trial is to assess non-inferiority of MVA-BN® compared to ACAM2000® in terms of PRNT antibody response at the Peak Visits (Day 42 for Group 1 and Day 28 for Group 2).

Suppose m1 is the PRNT $\log_{10}$ titer mean in Group 1 and m2 is the PRNT $\log_{10}$ titer mean in Group 2. The test of non-inferiority will be applied for the following hypothesis:

H0: $m1 - m2 \leq -\Delta$ versus H1: $m1 - m2 > -\Delta$ where

Δ is the non-inferiority margin. For the PRNT the non-inferiority margin will be 0.301 on the $\log_{10}$ scale which is equivalent to a doubling on the original titer scale for the GMT. This is the same Δ for the PRNT as was used for the assessment of non-inferiority of ACAM2000® to Dryvax® (ACAM2000 VRBPAC Briefing Document, April 2007).

The above hypotheses will be tested for the PRNT using a t-test on the difference of the two means based on the assumption that the $\log_{10}$ titers are normally distributed (which has been the case in all previous MVA-BN® studies). Specifically, a one-sided 97.5% confidence interval for the difference of the $\log_{10}$ titer means (based on the assumption of a common standard deviation [SD]) will be calculated. If the lower limit of this confidence interval is greater than $-\Delta$, then the null hypothesis will be rejected and non-inferiority of MVA-BN® to ACAM2000® will have been demonstrated.

For better interpretation the primary analysis will be presented in terms of the ratio of the GMTs, which is equivalent to taking the antilog of the differences in the $\log_{10}$ means. Similarly the corresponding confidence intervals are presented on the original titer scale by taking the antilog of the CIs calculated on the $\log_{10}$ scales. In this way, non-inferiority of MVA-BN® to ACAM2000® will be demonstrated if the lower limit of CI of the ratio of the GMTs lies within the interval above ½. This interval is obtained as the antilog of the $-\Delta$.

A similar analysis will be conducted for descriptive purposes on the ELISA $\log_{10}$ titer means. This will be done as secondary endpoint. The non-inferiority margin is chosen to be 0.176 for the ELISA on the $\log_{10}$ scale (which is equivalent to a factor of ⅔ on the original titer scale for the GMT).

All immunogenicity results will be listed. Tables and figures will be prepared for the PPS, ISS and the FAS. All of the following analyzes will be performed for results generated using both the PRNT and ELISA.

Seropositivity rates

Vaccinia-specific seropositivity rates will be presented by group for all sampling visits along with the Clopper-Pearson 95% confidence intervals (CIs) of the seropositivity rates. In addition, the Fisher's exact test will be performed between groups to determine if there is any difference in seropositivity rates.

Seroconversion rates

Vaccinia-specific seroconversion rates will be presented by group for all post baseline sampling visits along with the Clopper-Pearson 95% CIs of the seroconversion rates. In addition, the

Fisher's exact test will be performed between groups to determine if there is any difference in seroconversion rates.

Serovconversion rates will be presented graphically in a bar chart by visits differentiated by groups.

Geometric Mean Titer

GMTs will be calculated at all sampling visits for each group. GMTs and upper and lower confidence limits will be displayed to one decimal place.

Descriptive statistics will be derived by visit including number of observations available at each visit (n), GMT, with 95% CI (derived by the antilogarithm of the 95% CI of the $\log_{10}$ titer transformations).

The ratio of the GMTs will be presented between groups (Group 1 / Group 2) for Peak Visits along with the 95% CI. This ratio will be used to perform the non-inferiority analysis for the Peak Visit GMTs.

GMTs will be presented graphically in a line chart by visits differentiated by groups. The GMT will be displayed on the Y axis using a base 10 exponential axis, while visits will be displayed on the X axis by week.

Correlation of ELISA and PRNT results

Pearson's correlation coefficient (with associated CI) for $\log_{10}$ titers measured by ELISA and PRNT at each post baseline sampling visit will be calculated per group with the associated p-values and 95% CIs for the PPS, ISS and FAS. The correlation will also be presented on a scatter plot by group.

3.10.8 Efficacy Analysis

The co-primary efficacy endpoint of the trial is the MLA in mm^2 after scarification with ACAM2000. The primary analysis assesses if the AAR (based on the MLA) is significantly above λ=40% using the HL method for calculating the non-parametric confidence interval as described in Section 3.3 and implemented using the HL option in the SAS procedure NPAR1WAY.

In addition, the median for each group (and overall for both groups) will be calculated along with the 95% non-parametric confidence intervals for the median (Hahn, 1991). This will be obtained using the SAS Univariate procedure with the option CIPCTLDF. In addition, the minimum and maximum values will be displayed.

A similar secondary supportive analysis will be performed using the PB method (described in Appendix 6.1).A similar secondary descriptive analysis will also be performed to calculate the confidence interval of the AAR (based on the MLD) using the HL method for:

- lesion area (mm^2) at Day 6-8
- lesion area (mm^2) at Day 13-15

and the DAR using the investigator measurements of:

- maximum lesion diameter (mm)
- lesion diameter (mm) at Day 6-8
- lesion diameter (mm) at Day 13-15

3.10.9 Adverse Events

Adverse Events

All AEs recorded in the screening period will be reported as baseline signs and symptoms.

A summary table will be presented of the number (and percentage) per vaccination period both per subject and for the number of events for the following events:

- Any AEs;
- Any treatment emergent AEs (i.e. onset date within 28 days after vaccination); note all SAEs are automatically considered treatment emergent regardless of onset date-
- Any Causally Related AEs (i.e. missing, unknown, not evaluable, possibly, probably, or definitely related AE); note that all local solicited AEs are automatically considered casually related;
- Any Causally related Treatment Emergent AEs;
- Treatment Emergent Severe AEs (Grade 3 or higher, for grading please refer to the CTP);
- Severe Causally related treatment emergent AEs);
- Non-serious Treatment Emergent AEs;
- SAEs;
- AESIs;
- AEs leading to discontinuation of treatment

In addition, the incidence of AEs will be calculated (i.e. the number of subjects developing a treatment emergent AE divided by the number of subjects at the time of the vaccination) with corresponding 95% Clopper-Pearson CI. The incidence of any AE will also be calculated for each vaccination period, overall and per subject.

For analysis the information will be based on the data provided from Screening Visit up until FU Visit.

All AEs will be listed by subject, including demographic information, SOC and PT.

According to the CTP no new AEs should be reported during the FU phase, except SAEs and AESIs, but if any are reported then they will be listed but considered non-treatment emergent.

The number of subjects with at least one treatment emergent AE will be compared between the groups by means of Fisher's exact test.

Serious Adverse Events

The number of subjects with at least one SAE will be compared between the groups by means of Fisher's exact test.

SAEs will be listed in the same manner as AEs.

Adverse Events of Special Interest

AESIs will be reported separately but in the same way as SAEs.

Solicited Local AEs

Solicited local AEs will be summarized by PT after each vaccination, and broken down by maximum intensity.

In addition, the incidence will be calculated by severity (i.e. the number of subjects developing a particular local solicited AE during the 15-day period after each vaccination divided by the number of subjects at the time of the vaccination) with corresponding 95% Clopper-Pearson CI. The incidence will also be calculated for any solicited local AE for each vaccination period and per subject.

The duration of the AEs will be included in the listing. The duration of the AEs will also be summarized using the mean, SD, median, minimum and maximum.

Solicited General AEs

Solicited general AEs will be summarized in the same manner as solicited local AEs.

In addition a similar summary table will be produced for relationship of the AEs to trial vaccine. Also the incidences of causally related AEs, and those with casually related with Grade ≥ 3 will be summarized per vaccination period and overall by subject.

Unsolicited AEs

Treatment emergent unsolicited AEs will be summarized by SOC and PT and vaccination period and by subject.

A summary of the number of AEs by intensity and by relationship to trial vaccination will also be presented.

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

Non-treatment emergent AEs will only be flagged and included in the listing, but will not be included in the tables.

Assessment of ACAM2000® Vaccination Site

The following additional four measurements will be derived from the subject's memory aids:

- maximum lesion diameter measured in mm
- maximum erythema measured in mm
- maximum induration measured in mm
- healing time (calculated using date scab fell off)

For descriptive purposes the median for each group (and overall for both groups) will be calculated along with non-parametric confidence intervals for the median (Hahn, 1991). This will be obtained using the SAS Univariate procedure with the option CIPCTLDF. In addition the minimum and maximum value will be displayed.

The medians in Group 1 and Group 2 will be presented along with the 95% non-parametric PB confidence interval of the ratio.

The investigators measurement of the lesion size, erythema and induration will be presented in a similar way for the Day 7, Day 14 and Day 28 post ACAM2000® vaccination.

In addition, a blinded ITRC will classify individual takes as either full, partial or absent. The details of how the ITRC makes the classification will be described in a separate ITRC Charter. Clopper-Pearson confidence intervals will be calculated for the proportions of subjects with either full, partial and absent takes as classified by the ITRC.

3.10.10 Clinical Laboratory Variables (Hematology, Chemistry)

All measured hematology and chemistry values (and changes from baseline for continuous parameters) will be listed and summarized at each visit using descriptive statistics.

Summary tables will be produced for the number of high and low laboratory values at each visit by hematology and chemistry parameters.

In addition, all clinical laboratory values outside the normal range will be listed with screening number, demographic information and flagging of abnormal values (L = Below normal range, H = Above normal range) with their clinical significance.

'Shift tables' will be used to evaluate categorical changes from SCR to Visit 3, SCR to Visit 6 (Group 1 only) and SCR to Visit 9 (Group 1 only) with respect to normal ranges (below, within, above normal range) in hematology and chemistry parameters.

3.10.11 Vital Signs and ECG

Measured vital signs values and changes from Baseline will be summarized at each time point using descriptive statistics. All measured values will be listed.

The investigator's overall interpretation of either normal, abnormal and if abnormal whether the ECG is clinically significant or not clinically significant, will be summarized at Screening and Visit 3. All ECGs including those clinically indicated and those not clinically indicated will be listed. In addition shift tables between SCR and Visit 3 and between SCR and Visit 9 (Group 1 only) will be produced to summarize the number of changes in ECG status.

3.10.12 Pregnancy Test

Results of pregnancy tests will be presented in an individual subject listing.

3.10.13 Physical Examination

Subjects with abnormal physical examinations will be listed.

4 References

ACAM2000 Vaccines and Related Biological Products Advisory Committee (VRBPAC) Briefing Document, April 2007: http://www.fda.gov/ohrms/dockets/ac/07/briefing/2007-4292B2-00-index.htm

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5 Description of Appendix Tables and Listings

If not stated otherwise, data are described in the following way:

Summary descriptive statistics for continuous data: n, arithmetic mean, standard deviation, minimum, maximum, median, lower and upper 95% confidence limits where appropriate.

Frequency tables for categorical data: display of the sample used, absolute numbers and percentage for each category and lower and upper 95% confidence limits where appropriate.

5.1 Tables and Figures

Section 15.1 Disposition and Demographics

15.1.1 Disposition
15.1.1.1 Eligibility of the Subject and Analysis Sets – All Subjects
15.1.1.2 Reason for Withdrawal from 2nd Vaccination/3rd Vaccination – FAS (Group 1 only)

15.1.2 Demographic Data
15.1.2.1 Demographic Data – FAS
15.1.2.2 Demographic Data stratified by Gender – FAS
15.1.2.3 Demographic Data – PPS
15.1.2.4 Demographic Data – ISS
15.1.2.5 Cardiac Risk Assessment – FAS

15.1.3 Medical History
15.1.3.1 Medical History by Preferred Term and System Organ Class – FAS

15.1.4 Prior and Concomitant Medication
15.1.4.1 Prior Medication, by ATC Class and Generic Name – FAS
15.1.4.2 Concomitant Medication, by ATC Class and Generic Name – FAS

15.1.5 Compliance
15.1.5.1 Compliance: Number of Subjects who Received Vaccinations – FAS

Section 15.2 Efficacy and Immunogenicity

15.2.1 Assessment of Take following ACAM2000® Vaccination
15.2.1.1.1 Statistics of SilhouetteConnect camera Lesion Area after ACAM2000® Vaccination – PPS
15.2.1.1.2 Statistics of SilhouetteConnect camera Lesion Area after ACAM2000® Vaccination – FAS
15.2.1.1.3 Statistics of SilhouetteConnect camera Lesion Area after ACAM2000® Vaccination – ISS

15.3.3.1.1 ECG Data: Global Information – FAS
15.3.3.1.2 ECG Data (Shift Table): Global Information – FAS
15.3.3.1.3 ECG Data: Investigator Assessment – FAS

15.3.3.2 Vital Signs – FAS

15.3.3.3 Laboratory Data

15.3.3.3.1 Laboratory Data (Mean Values): Hematology – FAS
15.3.3.3.2 Laboratory Data (Mean Values): Biochemistry – FAS
15.3.3.3.3 Laboratory Abnormalities (Summary Table): Hematology – FAS
15.3.3.3.4 Laboratory Abnormalities (Summary Table): Biochemistry – FAS

15.3.3.3.5 Laboratory Abnormalities (Summary Table): Troponin I – FAS
15.3.3.3.6 Laboratory Data (Shift Table): Hematology – FAS
15.3.3.3.7 Laboratory Data (Shift Table): Biochemistry – FAS

5.2 Listings

Section 16.2.1 Disposition and Demographics

16.2.1.1 Disposition
16.2.1.1.1 Eligibility for Trial Participation – All Subjects
16.2.1.1.2 Violation of Inclusion Criteria – All Subjects
16.2.1.1.3 Exclusion Criteria Fulfilled – All Subjects
16.2.1.1.4 Overall Assessment – All Subjects
16.2.1.1.5 Withdrawal from Second or Third Vaccination – FAS (Group 1 only)
16.2.1.1.6 Disposition – FAS

16.2.1.2 Demographic Data
16.2.1.2.1 Demographic Data – All Subjects
16.2.1.2.2 Cardiac Risk and Smallpox Vaccination History – All Subjects

16.2.1.3 Medical History
16.2.1.3.1 Medical History – FAS
16.2.1.3.2 Baseline Signs and Symptoms – FAS
16.2.1.3.3 Abnormal Physical Examination at Screening – FAS

16.2.1.4 Prior and Concomitant Medication
16.2.1.4.1 Prior Medication – FAS
16.2.1.4.2 Concomitant Medication – FAS

16.2.1.5 Compliance
16.2.1.5.1 Dates of Visits and Blood Sampling – FAS
16.2.1.5.2 Investigational Product Administration – FAS

16.2.1.5.3 Memory Aid Accounting – FAS

Section 16.2.2 Efficacy and Immunogenicity

16.2.2.1 Measurement of Lesion Area and Diameter using the SilhouetteConnect camera system – FAS

16.2.2.2 ITRC Assessment of lesion following vaccination with ACAM2000® – FAS
16.2.2.3 Vaccinia-Specific PRNT Antibody Analysis: Titers, Seropositivity and Seroconversion Status – FAS
16.2.2.4 Vaccinia-Specific ELISA Antibody Analysis: Titers, Seropositivity and Seroconversion Status – FAS

Section 16.2.3 Safety

16.2.3.1 Adverse Events
16.2.3.1.1 Unsolicited Adverse Events
16.2.3.1.1.1 Unsolicited Adverse Events – FAS
16.2.3.1.1.2 Related Unsolicited Adverse Events – FAS
16.2.3.1.1.3 Grade ≥3 Unsolicited Adverse Events – FAS
16.2.3.1.1.4 Related Grade ≥3 Unsolicited Adverse Events – FAS

16.2.3.1.2 Solicited Local Adverse Events
16.2.3.1.2.1 Vaccination Site Local Symptoms – FAS

16.2.3.1.2.2 Duration of Solicited Local Adverse Events – FAS
16.2.3.1.2.3 Vaccination Site Appearance – FAS

16.2.3.1.3 Solicited General Adverse Events
16.2.3.1.3.1 Solicited General Symptoms – FAS
16.2.3.1.3.2 Duration of Solicited General Adverse Events – FAS
16.2.3.1.3.3 Duration of Related Solicited General Adverse Events – FAS
16.2.3.1.3.4 Swollen Lymph nodes – FAS

16.2.3.1.4 Assessment of ACAM2000® Vaccination Site
16.2.3.1.4.1 Investigator Assessment of Lesion Diameter, Erythema, Induration and General Appearance following vaccination with ACAM2000® – FAS
16.2.3.1.4.2 Subject memory aid measurement of Maximum Erythema, Induration and Lesion Diameter following vaccination with ACAM2000® – FAS

16.2.3.2 Serious Adverse Events

16.2.3.2.1 Serious Adverse Events – FAS
16.2.3.2.2 Related Serious Adverse Events – FAS
16.2.3.2.3 Adverse Events of Special Interest – FAS
16.2.3.2.4 Related Adverse Events of Special Interest – FAS
16.2.3.2.5 Adverse Events of Special Interest: Cardiac Follow-up – FAS

16.2.3.3 Laboratory Data, Vital Signs and ECG data

16.2.3.3.1 Laboratory Data

16.2.3.3.1.1 Laboratory Data: Hematology – FAS
16.2.3.3.1.2 Laboratory Data: Hematology – Abnormal Values
16.2.3.3.1.3 Laboratory Data: Biochemistry – FAS
16.2.3.3.1.4 Laboratory Data: Biochemistry – Abnormal Values
16.2.3.3.1.5 Laboratory Data: Pregnancy Test Results (Female Subjects of Childbearing Potential Only) – FAS
16.2.3.3.1.6 Laboratory Data: Laboratory Normal Ranges

16.2.3.3.2 Vital Signs – FAS

16.2.3.3.3 ECG Data

16.2.3.3.3.1 ECG Data: Investigator Assessment – FAS
16.2.3.3.3.2 ECG Data: Central Assessment – FAS

16.2.3.3.4 Physical Examinations

16.2.3.3.4.1 Abnormal Targeted Physical Examination – FAS
16.2.3.3.4.2 Lymphadenopathy – FAS

6 Appendices

6.1 Appendix 1: SAS code for POX-MVA-002 Example

```
/* POX-MVA-002 lesion diameter data for Groups T1 and T2 */
data POX002; input SUBJECT$ T$ MLD @@; datalines;
[redacted]  T2  2   [redacted]  T2  7   [redacted]  T2  8
[redacted]  T2  10  [redacted]  T2  7   [redacted]  T2  9
[redacted]  T2  7   [redacted]  T2  7   [redacted]  T2  4
[redacted]  T2  5   [redacted]  T2  0   [redacted]  T2  0
[redacted]  T1  10  [redacted]  T1  15  [redacted]  T1  20
[redacted]  T1  10  [redacted]  T1  20  [redacted]  T1  13
[redacted]  T1  12  [redacted]  T1  9   [redacted]  T1  10
[redacted]  T1  10  [redacted]  T1  11  [redacted]  T1  17
[redacted]  T1  13
; run;

data G1 G2; set POX002; if T="T2" then output G1; else output G2; run;

data G1; set G1; do i = 0 to _N_; MLD=MLD + i/1000000; end; run;
proc sort data=G1; keys MLD; run;                /* Sort dataset  */
proc rank data=G1 out=G1R; var MLD; ranks R; run;   /* Assigns unique Ranks */

data G1; set G1R; /* Calculate log values */
   if MLD = 0 then MLD = 1;
   MLDSTAR = log(MLD);
   MLA = MLD*MLD;
   MLASTAR = log(MLA);
   G=1;
run;

data G2; set G2; do i = 0 to _N_; MLD=MLD + i/1000000; end; run;
proc sort data=G2; keys MLD; run;                /* Sort dataset  */
proc rank data=G2 out=G2R; var MLD; ranks R; run;     /* Assigns unique Ranks */

data G2; set G2R; /* Calculate log values */
   if MLD = 0 then MLD = 1;
   MLDSTAR = log(MLD);
   MLA = MLD*MLD;
   MLASTAR = log(MLA);
   G=2;
run;

data G; set G1 G2; run;
```

```
/* Find number of observations in G1 and median */
proc means data=G1 noprint; var MLA; output out=C1 N=N1 median=M1; run;
data C1; set C1;
  C1=round((N1+1)/2-sqrt(N1),1);
  P1 = CDF('BINOM',c1-1,0.5,n1);
  Z1=quantile("Normal", (1-P1));
  G=1;
  keep C1 N1 P1 Z1 M1 G;
run;

data G1; merge G1 C1; by G; run;
data YC1; set G1; if R=C1; YC1=MLASTAR; UC1=MLDstar; run;
data YN1; set G1; if R=N1-C1+1; YN1=MLASTAR; UN1=MLDstar; run;
data Y1; merge YC1 YN1; keep YN1 YC1 UN1 UC1 Z1 M1; run;

/* Find number of observations in G2 and median */
proc means data=G2 noprint; var MLA; output out=C2 N=N2 median=M2; run;
data C2; set C2;
  C2=round((N2+1)/2-sqrt(N2),1);
  P2 = CDF('BINOM',c2-1,0.5,n2);
  Z2=quantile("Normal", (1-P2));
  G=2;
  keep C2 N2 P2 Z2 M2 G P2;
run;

data G2; merge G2 C2; by G; run;
data YC2; set G2; if R=C2; YC2=MLASTAR; UC2=MLDstar; run;
data YN2; set G2; if R=N2-C2+1; YN2=MLASTAR; UN2=MLDstar; run;
data Y2; merge YC2 YN2; keep YN2 YC2 UN2 UC2 Z2 M2 P2; run;

data Y; merge Y1 Y2;
  label M1 = “Median Group 1” M2 = “Median Group 2”;
  MLA_Var1 = ((YN1-YC1)/(2*Z1))**2;
  MLA_Var2 = ((YN2-YC2)/(2*Z2))**2;
  MLD_Var1 = ((UN1-UC1)/(2*Z1))**2;
  MLD_Var2 = ((UN2-UC2)/(2*Z2))**2;
  MLA_MR = (1+M1)/(1+M2);
  MLD_MR = (1+sqrt(M1))/(1+sqrt(M2));
  MLA_V  = exp(1.96*sqrt(MLA_Var1+MLA_Var2));
  MLD_V  = exp(1.96*sqrt(MLD_Var1+MLD_Var2));
/* confidence interval of AAR */
  AAR_UR = 1-(MLA_MR/MLA_V);
  AAR_LR = 1-(MLA_MR*MLA_V);
/* confidence interval of DAR */
  DAR_UR = 1-(MLD_MR/MLD_V);
```

```
  DAR_LR = 1-(MLD_MR*MLD_V);
  DAR = 1-MLD_MR;
  AAR = 1-MLA_MR;
run;

title "AAR & DAR with 95% PB CI";
proc print data=Y noobs labels;
   var M1 M2 AAR AAR_LR AAR_UR DAR DAR_LR DAR_UR;
run;
```