Statistical Analysis Plan

POX-MVA-031

A randomized, double-blind, multicenter Phase III trial to evaluate immunogenicity and safety of three consecutive production lots of a freeze-dried formulation of MVA-BN® smallpox vaccine in healthy, vaccinia-naïve subjects

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Abbreviations

Table 1: Abbreviations and Definitions

Abbreviation	Definition
AE	Adverse Event
AESI	Adverse Event of Special Interest
ADR	Adverse Drug Reaction
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ATC	Anatomic-Therapeutic-Chemical
β-HCG	Beta-Human Chorionic Gonadotropin
BMI	Body Mass Index
CI	Confidence Interval
CSR	Clinical Study Report
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ELISA	Enzyme-Linked Immunosorbent Assay
FAS	Full Analysis Set
FCS	Fully Conditional Specification
FD	Freeze Dried
FU	Follow Up
GMT	Geometric Mean Titer
ICF	Informed Consent Form
ICH	International Council for Harmonisation
IMP	Investigational Medicinal Product
LLOQ	Lower Limit of Quantitation
MedDRA	Medical Dictionary for Regulatory Activities
MI	Multiple Imputation
mL	Milliliters
MP	Medicinal Product
MVA-BN	Modified Vaccinia Ankara-Bavarian Nordic
PMM	Predictive Mean Matching
PPS	Per Protocol Set
PRNT	Plaque Reduction Neutralization Test
PT	Preferred Term
RBC	Red Blood Cell/Erythrocyte
RTSM	Randomization and Trial Supply Management (Medidata RAVE product)
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SC	Subcutaneous
SD	Standard Deviation
SDTM	Study Data Tabulation Model
SI	Système International d'Unités
SOC	System Organ Class
SOP	Standard Operating Procedure
ULN	Upper Limit of Normal
WBC	White Blood Cell/Leukocyte

Abbreviation	Definition
WHO	World Health Organization
WHO Drug	World Health Organization Drug Dictionary
WOCBP	Woman of Childbearing Potential

General Definitions

Vaccination

A vaccination in this trial means vaccination with any active trial vaccine, Modified Vaccinia Ankara-Bavarian Nordic (MVA-BN), regardless of lot number.

Randomized Subjects

A subject is considered randomized once they have been allocated a randomization number, regardless of whether or not they have received a vaccination.

Vaccinated Subjects

A subject is considered vaccinated once they have received the first vaccination.

Study Day

The day of study is defined from the day of the first vaccination. The day of first vaccination is defined as Day 1 and the day after first vaccination is Day 2, and so on. Equally, the day before first vaccination in the screening period 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 study day, i.e., at midnight a new study day begins regardless of the time of first vaccination.

Baseline

If not otherwise specified, "baseline" refers to the last measurement prior to first vaccination, including re-screening or pre-vaccination unscheduled visit values. These data will be summarized as baseline data without a specification of from which visit the data were derived. If data are collected on the day of first vaccination without a corresponding time, they will be considered for inclusion as baseline values if they were scheduled to occur prior to the vaccination at that visit. In cases where no pre-vaccination values exist for a baseline parameter, the subject will be considered as having no baseline and post-baseline changes will not be calculated.

Screening Phase

All data collected from the Screening visit until first vaccination. This could include any unscheduled visits or re-screening visits up to the first vaccination.

Active Trial Phase

The period starting at Visit 1 (the visit of first vaccination) through Visit 5 (28-35 days post second vaccination). Note for baseline calculation purposes, data that were to be collected prior to the first vaccination, but on the same day of first vaccination per the protocol schedule of events, will be considered pre-vaccination data even though they fall in the active trial phase.

Vaccination Period 1

Date/time of 1st vaccination until date/time before the 2nd vaccination or Day 35 (upper limit of the Visit 5 window) if the 2nd vaccination date is missing.

Vaccination Period 2

The period from the second vaccination through the latest of 35 days post-second vaccination or the date of Visit 5.

Overall Vaccination Period

The period from the first vaccination through the last vaccination + 35 days or the date of Visit 5, whichever is later. Note, due to the safety Adverse Event (AE) collection policy in the clinical trial protocol, Serious Adverse Events (SAEs) and Adverse Events of Special Interest (AESIs) are collected through the follow-up period and will not be reported as part of the Overall Vaccination Period.

Follow-up (FU) Period

All data collected after the Active Trial Phase until the final FU contact, scheduled to be 182 to 210 days after the last vaccination based on the protocol. Adverse events collected between Visit 5 (or 35 days post-last vaccination) and the FU visit will be considered part of the FU Phase.

Withdrawal From Second Vaccination

Subjects who receive the first vaccination but withdraw prior to receiving the second vaccination will be considered withdrawals from second vaccination. The reason for discontinuation on the trial disposition form will be used as their reason for discontinuation from second vaccination.

Withdrawal from Trial

Subjects who are randomized but withdraw prior to Visit 5 are considered withdrawals from the trial. The reason for discontinuation on the trial disposition form will be used as their reason for discontinuation from the trial.

Medical History

Diseases, treatments, and surgical interventions present before and up to signing of informed consent form (ICF) will be considered medical history. Medical history will be collected and documented in the Medical History electronic case report form (eCRF) page at screening.

Adverse Events

- **Unsolicited AEs**

AEs defined as any untoward (undesirable) occurrence of a medical event in a clinical trial subject temporally associated with the administration of an investigational medicinal product (IMP) or a medical product (MP) which does not necessarily have a causal relationship with this IMP/MP. Up to Visit 5 all AEs (e.g. feeling of ill-health, subjective symptoms and objective signs, intercurrent diseases, accidents, etc.) observed by the investigator and/or reported by the subject must be recorded in the eCRF and in the subject's medical record regardless of the assessment of causality in relationship with the IMP/MP. In addition, SAEs and AESIs are collected through the FU Period, between Visit 5 and the FU Visit.

Abnormal laboratory values assessed as being clinically significant by the investigator are to be documented as AEs. In addition, abnormal laboratory values fulfilling the Grade 3 or Grade 4 criterion according to the toxicity scale (see Clinical Trial Protocol Appendix 1) are to be documented as AE in the eCRF and in the subject's medical record, regardless of whether they are considered clinically relevant or not. Toxicity grade and seriousness of an AE will be assessed separately, i.e., a Grade 3 or Grade 4 AE will not automatically be regarded as serious.

- **Solicited AEs**

  All symptoms specifically listed in the memory aid provided to the subjects following each vaccination are considered Solicited AEs. The subjects are requested to monitor and record local symptoms in the memory aid, i.e., erythema, swelling, induration, pruritus and pain at the site of injection as well as general symptoms, i.e., body temperature, headache, chills, myalgia, nausea and fatigue daily for the day of vaccination and the following 7 days (Days 1-8, 8-day duration).

Adverse Drug Reaction (ADR)

An AE determined to be related to vaccination, defined as an investigator assessment of causality as possible, probable, or definite. ADRs are also referred to as related AEs. Solicited local AEs are automatically considered related to vaccination, however causality for solicited general AEs will be determined by the investigator. In the case of missing causality assessments, an AE will be considered related for analysis purposes.

Serious Adverse Events

An AE that meets one or more of the seriousness criteria as described in the trial protocol.

Adverse Events of Special Interest

An AESI is defined in this trial as:

- Any cardiac sign or symptom developed since the first vaccination
- ECG changes determined to be clinically significant
- Cardiac enzyme troponin I > Upper Limit of Normal (ULN; ≥ Grade 1)

All AESIs recorded with onset after the first trial vaccination through Visit 5 will be included in the Overall Vaccination Period, and those collected from Visit 5 through the FU visit are considered part of the FU Period.

Lower Limit of Quantitation

The lower limit of quantitation (LLOQ) is defined as follows:

- Enzyme-Linked Immunosorbent Assay (ELISA) titer of 200
- Plaque Reduction Neutralization Test (PRNT) titer of 20

Values below the LLOQ will be imputed with 1/2 of the LLOQ, 100 for the ELISA and 10 for the PRNT.

Seroconversion

Seroconversion is defined as either the appearance of antibody titers ≥ LLOQ for subjects with a titer below LLOQ at baseline, or a doubling (or more) of the antibody titer compared to the baseline titer for subjects with a titer equal or above the LLOQ at baseline.

Geometric Mean Titer (GMT)

The GMT is calculated by taking the antilogarithm of the mean of the $\log_{10}$-transformed titers.

1 Introduction

This Statistical Analysis Plan (SAP) details the statistical methodology to be used in analyzing trial data and outlines the key statistical programming specifications. It describes the variables and populations, anticipated data transformations and manipulations, and other details of the analyses not provided in the trial protocol. The SAP is written based on recommendations from *ICH E3: Structure and Content of Clinical Study Reports*, and *ICH E9: Statistical Principles for Clinical Trials*. Table, figure, and listing shells are described in a separate document.

The included analyses described are based on the final clinical trial protocol *A randomized, double-blind, multicenter Phase III trial to evaluate immunogenicity and safety of three consecutive production lots of a freeze-dried formulation of MVA-BN® smallpox vaccine in healthy, vaccinia-naïve subjects, Edition 3.0*, dated 22 November 2019. This SAP will be followed completely for the analysis of data derived from the clinical trial. If any unforeseen additional analyses are included in the clinical study report (CSR) they will be clearly described as additional, unplanned analyses.

2 Trial Overview

2.1 Trial Description

The POX-MVA-031 trial is a randomized, double-blind, multicenter Phase III trial to evaluate immunogenicity and safety of three consecutive production lots of a freeze-dried formulation of the MVA-BN smallpox vaccine in healthy, vaccinia-naïve subjects.

In total, approximately 1110 vaccinia-naïve subjects are to be enrolled in this trial. All subjects will be randomly assigned (1:1:1) to one of three MVA-BN groups to receive two vaccinations each with FD MVA-BN administered in a double-blind manner.

Group 1: 370 subjects will receive two SC vaccinations with 0.5 mL FD MVA-BN Lot 1

Group 2: 370 subjects will receive two SC vaccinations with 0.5 mL FD MVA-BN Lot 2

Group 3: 370 subjects will receive two SC vaccinations with 0.5 mL FD MVA-BN Lot 3

2.2 Design Techniques to Avoid Bias

POX-MVA-031 will be performed as a double-blind trial in which neither the subject nor the staff performing the trial will be aware of the MVA-BN lot group to which the subject was assigned. Randomization will ensure that any differences in baseline characteristics, if they occur, will be purely due to chance and not due to bias in group assignment.

2.2.1 Methods of Assigning Subjects to Treatment Groups

Randomization to vaccine lot will be performed at Visit 1, after re-confirmation of each subject's eligibility and all procedures required prior to first vaccination have been performed. The randomization ratio is 1:1:1, such that approximately the same number of subjects are assigned to each of the MVA-BN lots. Randomization will be stratified by clinical trial site, as well as blocked within site to reduce the chance of a site-level imbalance in subject assignment to each of the three lots. Medidata Randomization and Trial Supply Management System (RTSM) will be used to perform a centralized randomization, as well as to assign the appropriate MVA-BN lot to subjects at Visits 1 and 3. Medidata RTSM will be integrated with Medidata RAVE, the electronic database capture system, such that the site is required to confirm eligibility before a randomization number is assigned and any MVA-BN product is dispensed.

2.2.2 Blinding

The trial will be double-blinded, meaning that neither the subjects nor the staff running the trial will have knowledge of which MVA-BN lot the subject was assigned. In addition, the contract research organization, ICON, and the trial team at Bavarian Nordic (BN) will remain blinded until the completion of the trial. The few exceptions to this will be the unblinded site staff who prepare the vaccine, the unblinded clinical research associates who review the vaccine shipments and randomized lot assignments, and the unblinded trial supply staff at BN who:

- maintain the products at an appropriate level for each active site
- review Drug Delivery Notes and temperature monitoring data after each vaccine shipment to site to check that vaccine has been received at site undamaged and according to temperature specifications
- receive and manage the assessment of temperature excursion data relating to vaccine stored at site
- control the quarantine classification of vaccine at site through RTSM.

Blinding will be maintained using the Medidata RTSM system, which will only be accessible by unblinded trial staff.

2.3 Objectives

2.3.1 Primary Objective

To assess the consistency of three consecutively produced lots of a freeze-dried formulation of MVA-BN in terms of immunogenicity.

2.3.2 Secondary Objectives

The secondary objectives of the trial are:

- To assess uncommon adverse reactions.
- To collect vaccinia-specific humoral immune response data.

2.4 Trial Population

The trial population will include healthy men and women who are 18 to 45 years of age. The intention of the trial is to enroll vaccinia-naïve subjects. Hence, persons over the age of 45, those with a smallpox vaccination scar, those having a known or suspected history of smallpox vaccination, and/or those with military service prior to 1991 or after January 2003 will be excluded from the trial population. A consultation including a physical examination and laboratory testing will be performed to ensure the potential subjects are eligible per the inclusion and exclusion criteria for the trial.

2.5 Inclusion Exclusion Criteria

A list of inclusion and exclusion criteria is provided in the trial protocol. Subjects are required to meet all of the inclusion criteria and none of the exclusion criteria to enter the trial.

2.6 Endpoints

2.6.1 Primary Endpoint

GMTs as measured by PRNT 2 weeks following the second vaccination.

2.6.2 Secondary Immunogenicity Endpoints

- GMTs as measured by ELISA 2 weeks following the second vaccination.
- PRNT seroconversion rates 2 weeks following the second vaccination.
- ELISA seroconversion rates 2 weeks following the second vaccination.
- Pearson's correlation coefficient between the $\log_{10}$ transformed PRNT titers and the $\log_{10}$ transformed ELISA titers 2 weeks following the second vaccination.

2.6.3 Secondary Safety Endpoints

- Occurrence, relationship and intensity of any SAEs at any time during the trial.
- Occurrence, relationship and intensity of any cardiac sign or symptom indicating a case of myo-/pericarditis at any time during the trial.
- Occurrence of any Grade 3 or 4 AEs probably, possibly, or definitely related to the trial vaccine within 28 days after each vaccination.
- Occurrence, relationship and intensity of unsolicited AEs within 28 days after each vaccination.

- Occurrence, intensity and duration of solicited local AEs (redness, swelling, induration, pruritus and pain) during the 8-day period (day of vaccination and the following 7 days) after each vaccination.
- Occurrence, relationship, intensity and duration of solicited general AEs (pyrexia, headache, myalgia, nausea, fatigue and chills) during the 8-day period (day of vaccination and the following 7 days) after each vaccination.

2.6.4 Interim Analysis

No interim analysis for the purpose of reviewing the primary immunogenicity results is planned for the trial.

Interim safety reviews will be performed by an independent Data and Safety Monitoring Board (DSMB). The DSMB will perform two data reviews during the course of the trial: after the first 555 subjects have completed Visit 2, and after all 1110 subjects have completed Visit 4. After the completion of the trial, the DSMB will review the full data for the trial. The DSMB will have the opportunity to review both blinded and unblinded (results broken down by MVA-BN lot) routine safety reports created specifically for the DSMB members by an external, independent programming team. BN and any blinded participants for open sessions of DSMB meetings will only be able to review the blinded routine safety reports.

Although the DSMB has the opportunity to review unblinded trial data, there will be no impact to the final hypothesis testing for the trial. The titer data which will be used for the primary hypothesis testing for the trial will not be available to the DSMB, as their purpose is only to review the safety and trial progress. No adjustments for type-1 error will need to be performed.

Details of the responsibilities and operation of the DSMB are located in the DSMB Charter.

3 Trial Design

Visit (V)	SCR	V1	V2	V3	V4	V5	FU Contact
Day / Visit +… D	**-28- -1**	**1**	**V1 +12-16**	**V1 +28-35**	**V3 +12-16**	**V3 +28-35**	**V3 +182-210**
Target week	**- 4**	**0**	**2**	**4**	**6**	**8**	**32**
Procedures							
Informed consent	X						
Inclusion/Exclusion criteria	X	X*					
Withdrawal criteria				X			
Medical History	X						
Smallpox vaccination/scar check	X						
Physical exam	X						
Vital signs	X	X	X	X	X	X	(X)[1]
Family cardiac risk factors	X						
Baseline signs and symptoms	X	X					
Targeted physical exam incl. auscultation of the heart and lung		X	X	X	X	X	(X)[1]
ECG[5]	X		(X)[2]		(X)[2]		
Prior/concomitant medications	X	X	X	X	X	X	
Pregnancy counseling for WOCBP[7]	X	X	X	X	X	X	
AE/SAE/AESI recording		X	X	X	X	X	X[3]
Laboratory Assessments							
Pregnancy test for WOCBP[4]	X	X		X		X	
Safety labs[5]	X		X		X		(X)[1]
Troponin I testing	X		(X)[2]		(X)[2]		(X)[2]
Antibody testing		X			X		
Vaccination							
Vaccine administration & subject observation (≥ 30 minutes)		X		X			

Visit (V)	SCR	V1	V2	V3	V4	V5	FU Contact
Day / Visit +… D	**-28- -1**	**1**	**V1 +12-16**	**V1 +28-35**	**V3 +12-16**	**V3 +28-35**	**V3 +182-210**
Target week	**- 4**	**0**	**2**	**4**	**6**	**8**	**32**
Immediate AEs		X		X			
Handout of memory aid		X		X			
Collection of memory aid			X		X		
Injection site exam			X		X		
Blood volume							
Appr. blood volume drawn (mL)[5, 6]	7	8.5	7	0	15.5	0	(7)[1]
Cumulative blood volume drawn (mL)[5]	7	15.5	22.5	22.5	38	38	(45)[1]

[1] If during the FU contact a serious condition is detected, the trial subject will be requested to return to the clinical trial site and the respective examinations will be performed.

[2] Only if clinically indicated, i.e., in the presence of cardiac signs or symptoms.

[3] New SAEs/AESIs and changes to SAEs/AESIs/AEs ongoing at V5 only.

[4] At Screening Visit, a serum test must be performed. At other visits, a urine pregnancy test will be performed.

[5] If clinically indicated, additional safety measures can be taken at any other trial visits or at unscheduled visits.

[6] Approximate amounts of single blood draws: Safety lab including all tests: 7 mL; antibody analysis: 8.5 mL

[7] Review of acceptable contraceptive methods and recent menstrual history with WOCBP.

(X) Only to be performed if clinically indicated.

* Once a subject has successfully met all eligibility requirements, then the subject will be randomized into the trial

4 Statistical Methods

4.1 Planned Sample Size

The primary objective of the trial is to demonstrate equivalence of three consecutively produced lots of MVA-BN in terms of the primary endpoint, the GMT measured by PRNT two weeks following the second vaccination.

In order to consider that small variations in the virus titer of the produced lots may influence the power to reject the null hypothesis (Ganju et al., 2008), a simulation study was performed to calculate the required number of analyzable subjects per group based on the following underlying assumptions:

- Significance level: 5% (two-sided), i.e., 95% CIs (two-sided)
- Power: 90%
- Within-lot variability in PRNT: $SD_{within} = 0.45$ on $\log_{10}$ transformed antibody titers (based on previous trials POX-MVA-006 and POX-MVA-013)
- Between-lot variability in PRNT: $SD_{between} = 0.075$ on $\log_{10}$ transformed antibody titers. This is derived as follows:
  - the SD of the dose of the produced lots is not larger than 0.13 (based on the upper 95% CI of $\log_{10}$ transformed virus titers observed in 8 released lots of MVA-BN FD)
  - the slope of the dose-response curve on the $\log_{10}$ scale is about 0.57 for the dose used in this trial (based on the data of trial POX-MVA-004)
  - these 2 assumptions translate to about 0.13x0.57 ~ 0.074 which was conservatively rounded to $SD_{between} = 0.075$
- Equivalence margin: Δ=0.301 on the $\log_{10}$ scale

The simulations showed that an analyzable sample size of 315 in each group yields a power of at least 90% of showing equivalence for all three MVA-BN groups.

In order to account for a dropout rate of approximately 15%, which has been observed in previous MVA-BN trials, a total of 370 subjects are planned to be randomized in each group.

4.2 Analysis Populations

For the purpose of analysis, subjects will be divided up into the following populations:

4.2.1 Full Analysis Set (FAS)

The FAS includes all subjects who were randomized and received at least one dose of trial vaccine, regardless of the occurrence of protocol deviations. Safety, demographics, and disposition analyses will be performed on this population.

4.2.2 Per Protocol Set (PPS)

The PPS is a subset of the FAS including all subjects without major protocol deviations that might have a substantial impact on the immunogenicity results used for the primary endpoint analysis.

The following deviations will be considered reasons to exclude subjects from the PPS:

- No Visit 4 immunogenicity result available
- > 72 hours turn around time from Visit 4 immunogenicity sample drawn to aliquoting
- < 15 minutes clotting time for Visit 4 immunogenicity sample
- Received < 2 vaccinations
- Violations of inclusion or exclusion criteria that will affect the primary immunogenicity outcome (per recurring protocol deviation reviews)
- Second vaccination < 25 days or > 42 days after first vaccination
- Visit 4 immunogenicity sample drawn < 9 days or > 23 days after second vaccination
- Prohibited medications including,
    - Live vaccines within 30 days prior to or after trial vaccination
    - Killed/inactivated vaccines within 14 days prior to or after trial vaccination
    - Chronic systemic administration (>14 days) of > 5 mg prednisone (or equivalent)/day or any other immune-modifying within three months prior to administration of trial vaccine or after trial vaccination
    - Immunoglobulins and/or any blood products within three months prior to administration of trial vaccine or after trial vaccination
    - Investigational or non-registered products within 30 days prior to or after trial vaccination

The primary analysis of immunogenicity will be performed on the PPS. In particular, the primary trial hypothesis tested will be performed on this analysis set. All analyses of immunogenicity will be repeated for the FAS to assess the robustness of the results, however the results of the test on the PPS will determine the success of the trial.

4.3 Data Handling Conventions

4.3.1 Visit Windowing

No visit windowing will be performed for safety results. AEs will be mapped to a vaccination period for analysis purposes. Laboratory values will be summarized by out of range or highest toxicity grade within each planned visit and during the overall vaccination period.

Antibody testing for immunogenicity results is only performed on samples collected at Visit 1 and Visit 4. Only antibody samples drawn prior to first trial vaccination at Visit 1 may be counted as baseline values. As the primary analysis for immunogenicity endpoints occurs for the PPS, it is not expected that these results are derived from off-window visits for subjects in the analysis set. For immunogenicity analyses in the FAS, any post-second vaccination immunogenicity results will be counted as Visit 4 results to get a full accounting of available data. If a subject has more than one post-second vaccination immunogenicity sample, the value drawn closest to the target day (14 days post-second vaccination) will be used for the Visit 4 value. If two are the same number of days apart from the target date, the earlier will be used.

4.3.2 Handling of Dropouts or Missing Data

In general, no imputation schemes for missing values will be applied. All data will be listed and summarized as captured in the eCRF or transferred from external sources (e.g., central lab, ECG).

For the primary endpoint, GMTs as measured by PRNT two weeks following the second vaccination, the analysis population includes only subjects who have no major protocol deviations that would affect this value. However, for sensitivity the primary endpoint will be analyzed using the FAS to include any additional results from this population which were excluded from the primary analysis. In addition, a multiple imputation (MI) analysis will be performed for full accounting of FAS subjects. Details are provided in Section 4.4.5.1.

In order to assign AE and medications to the correct trial period, incomplete or missing start and end dates may need to be imputed. These imputations will be performed as follows:

For prior and concomitant medications, as well as AEs, imputation of partial start and end dates will be performed 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.

Imputed dates will only be used to assign AEs and concomitant medications to the vaccination periods within the active trial period. Original values will be displayed in subject-level listings.

4.3.3 Assignment of AEs to Vaccination Period

Each AE will be assigned to a vaccination period using date/time of vaccination and date/time of onset of AE:

- All AEs beginning before the first trial vaccination will be classified as baseline signs and symptoms belonging to the Screening period.
- All AEs starting from the first vaccination through prior to the second vaccination will be assigned to Vaccination 1 Period.
- All AEs starting from the second vaccination through the latest of the last vaccination + 35 days or Visit 5 will be assigned to the Vaccination 2 Period.
- All AEs starting from the first vaccination through the latest of the last vaccination + 35 days or Visit 5 will be assigned to the Overall Vaccination Period.
- All SAEs and AESIs starting after Visit 5 through the FU Visit will be included in the FU Period for analyses.

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

All solicited AEs recorded on the memory aids will be considered as part of their corresponding vaccination period, regardless of missing or partial dates on the memory aid.

4.3.4 General Consideration for AEs

The Medical Dictionary for Regulatory Activities (MedDRA) version 22.0 will be used to code AEs, medical history events, and baseline signs and symptoms.

Duration of a solicited AE in days is calculated as end date of AE – onset 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 of solicited AEs ongoing at the end of the trial, the duration will not be calculated.

4.4 Analysis and Presentation Methods

4.4.1 General Presentation Methods

All individual data entered in the eCRF, received from vendors (e.g., lab data, ECG data) and derived data will be listed in subject-level data listings. Listings will be sorted by lot group, subject, visit, and parameter based on the domain presented. Tables will be sorted by subgroup (if applicable, Section 4.4.8), parameter, and scheduled visit, as applicable.

All tables will be presented by treatment groups as follows:

- Group 1
- Group 2
- Group 3
- Overall (only for demographics, disposition, and safety)

With the exception of immunogenicity data, continuous measurements will be summarized using the number of observations, mean, SD, minimum, median, and maximum. Categorical data will be summarized using frequencies and percentages, unless otherwise stated. Means and medians will be presented to one decimal place more than the precision of the original data. Standard deviations will be presented to two additional decimal places, and minimums and maximums will be presented using the original precision. No more than 4 decimal places will be presented, regardless of the precision of the original data. Percentages will be presented to one decimal place.

For ELISA and PRNT titers descriptive statistics will be based on the number of observations, geometric means, and 95% confidence intervals (CIs). The proportion of subjects with titers equal or above the LLOQ and the proportion of subjects who achieve seroconversion will be presented using frequencies, percentages, and exact Clopper-Pearson 95% CIs.

GMTs will be calculated by taking the antilogarithm of the mean of the $\log_{10}$ titer transformations. Antibody titers below the LLOQ will be given a value of 1/2 of the respective LLOQ for the purpose of calculations. GMTs and upper and lower confidence limits will be displayed to one decimal place.

All statistical summaries and analyses of safety and immunogenicity data will be performed using SAS® version 9.3 or higher (SAS Institute, Cary, NC, USA).

Repeated assessments/measurements and unscheduled assessments/measurements will be presented in subject-level data listings. In case of any repeats of pre-vaccination values, the latest pre-vaccination values will be used for the baseline value.

4.4.2 Considerations for Multicenter Studies

POX-MVA-031 is a multicenter trial, with 12 planned sites and up to 15 potential sites participating in the trial. Assuming 1110 subjects and 12 sites, approximately 93 subjects are expected at each site (i.e., 31 subjects per lot group per site). Due to competitive enrollment, it is not expected that all sites will enroll the same number of subjects.

Disposition summaries will be performed for the whole trial, as well as by trial site. In addition, as site was the stratification factor for randomization, the primary immunogenicity analysis will be repeated within each site as a subgroup analysis.

4.4.3 Adjustments for Covariates

No adjustments for covariates will be performed.

4.4.4 Adjustments for Multiple Comparisons

No adjustments for multiple comparisons will be required, as success in the trial requires the equivalence margin to hold for all three comparisons (Group 1 vs. Group 2, Group 2 vs. Group 3, and Group 1 vs. Group 3). Additionally, no interim analyses including the primary endpoint will be performed.

4.4.5 Primary and Secondary Endpoint Analyses

4.4.5.1 Primary Endpoint

The primary objective of the trial is to show that the humoral immune responses elicited by three consecutively produced MVA-BN lots are statistically equivalent. Specifically, the aim of this equivalence trial is to show that the three lots of MVA-BN produce statistically equivalent titers at a specific time point (2 weeks post-second vaccination) for subjects who are vaccinated according to the standard regimen of 2 doses, 28 days apart.

The humoral immune response, as specified in the primary objective, is defined as the within-group GMT of the individual subject PRNT titers measured at Visit 4 (i.e., 2 weeks after the second vaccination). As the titers are log-normally distributed, the endpoint will be tested on the $\log_{10}$ scale. The primary endpoint will be met if the means of the $\log_{10}$ titers are equivalent within a pre-specified amount. This amount is called the margin of equivalence (Δ).

Suppose m_1 is the mean of the $\log_{10}$ titers in Group 1, m_2 is the mean of the $\log_{10}$ titers in Group 2 and m_3 is the mean of the $\log_{10}$ titers in Group 3. The test on equivalence will be applied for the following hypothesis:

$$H_0: |m_1-m_2| \geq \Delta \text{ OR } |m_1-m_3| \geq \Delta \text{ OR } |m_2-m_3| \geq \Delta$$

versus

$$H_A: |m_1-m_2| < \Delta \text{ AND } |m_1-m_3| < \Delta \text{ AND } |m_2-m_3| < \Delta$$

Δ is set at 0.301 for the $\log_{10}$ PRNT titers, equivalent to a factor of 2 for the GMT.

Assuming the $\log_{10}$ titers are approximately normally distributed, the above hypothesis will be tested based on the difference of the $\log_{10}$ titer means. Specifically, the two-sided 95% CI limits for the pair-wise differences in $\log_{10}$ titer means based on the t distribution will be calculated, assuming equal variances in the three lot groups. If all of the lower interval limits for the three lot-group differences are above $-\Delta$, and all of the upper limits for the three lot-group differences below Δ, the null-hypothesis will be rejected in favor of the alternative hypothesis of equivalence.

For ease of interpretation, the primary analysis will also 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 CIs are presented on the original titer scale by taking the antilog of the CIs calculated on the $\log_{10}$ scales. In this way, equivalence between two groups is demonstrated if the CI of the ratio of the GMTs lies within the interval [½, 2], which is the same as the CI of the differences on the $\log_{10}$ scale lying within [-0.301, 0.301].

Sensitivity Analyses

The primary analysis will be repeated on the FAS using all available data as a sensitivity analysis. Specifically, any post-baseline titer value will be used as the Visit 4 value, even if the sample was taken at a different visit or out of window. If a subject has more than one post-second vaccination immunogenicity sample, the value drawn closest to the target day (14 days post-second vaccination) will be used for the Visit 4 value. If two are the same number of days apart from the target date, the earlier will be used.

In addition, to account for all subjects in the FAS for the primary PRNT analysis, multiple imputation (MI) will be used to impute missing titer values at 2 weeks post-second vaccination. Assuming the missing titer values are missing at random and the post-baseline $\log_{10}$ titer values are normally distributed, MI will be used to create 100 complete datasets that will account for the random variability in these titer values. Baseline $\log_{10}$ PRNT values, year of birth, sex, and race will be used in the joint model to predict the $\log_{10}$ Visit 4 PRNT value. As the missing pattern of titer values is not monotone, i.e., subjects may be missing baseline (Visit 1), Visit 4, or both baseline and Visit 4 PRNT values, a fully conditional specification (FCS) method will be used. This method assumes the existence of a joint

distribution for all variables used to predict the missing values and allows the baseline log_{10} titer value to be imputed, if needed, to inform the missing post-baseline titer values.

As subjects are required to be vaccinia-naïve at screening, most baseline titers are expected to be below the LLOQ and are nominally set to a value half the LLOQ. Due to the high proportion of values below the LLOQ at baseline, the normality assumption is violated for the log_{10} titer values at this time point. Predictive mean matching (PMM) will be used to impute any missing baseline log_{10} titers. PMM imputes values randomly selected from the set of observed values such that the imputed values are more plausible due to the violation of the normality assumption for this continuous value.

After the imputation of any missing baseline titer values, the post-baseline PRNT log_{10} titer value will be imputed using the FCS regression method. Again, for the FAS analysis any post-baseline value will be used and will therefore not be imputed during the MI procedure. The post-baseline value will be imputed using a regression model simulated from the posterior predictive distribution of the parameters baseline log_{10} PRNT, year of birth, sex, and race.

After the creation of the 100 complete datasets, the mean log_{10} titer values for each arm along with their standard errors will be calculated within each imputation and combined over the 100 imputations. Similarly, the pairwise differences in the mean log_{10} titer values will be calculated, and these differences and associated standard errors will be combined over the 100 imputations. The combined results will be presented on the log_{10} scale using means and 95% CIs, as well as back transformed to present the GMTs for each arm, GMT ratios for each pair-wise comparison, and associated 95% CIs.

MI analyses will be performed in SAS using the PROC MI and PROC MIANALYZE procedures. See Appendix 1 for sample code for this analysis. A minimum of half of the LLOQ (10 for PRNT) will be set for imputed log_{10} titer values to correspond to the observed data range. In addition, a seed of 31031 will be used for the procedure.

Subgroup Analyses

In addition, the following subgroups of the PPS will be analyzed: Clinical Trial Site, Sex, Age Group (median split), Ethnicity, and Race Group (White vs. Other). GMTs will be presented within each subgroup, along with their 95% CIs.

4.4.5.2 Secondary Endpoints

Immunogenicity Endpoints

Immunogenicity Variables

Titer equal or above LLOQ (yes/no) at Visit 1 and Visit 4

- Vaccinia-specific neutralizing antibodies (PRNT)

- Total vaccinia-specific antibodies (ELISA)

Seroconversion (yes/no) at Visit 4

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

Antibody titers at Visit 1 and Visit 4

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

ELISA titers will be analyzed using the same method as for the primary endpoint, however no equivalence analysis will be performed.

PRNT and ELISA seroconversion rates at 2 weeks post-second vaccination will be derived based on the baseline and Visit 4 titers.

Pearson correlation coefficients between the $\log_{10}$ transformed PRNT titers and the $\log_{10}$ transformed ELISA titers 2 weeks following the second vaccination will be calculated.

Analyses

Geometric Mean Titer

GMTs will be calculated by taking the antilogarithm of the mean of the $\log_{10}$ titer transformations. Antibody titers below the LLOQ will be given a value of half of the LLOQ (i.e. 100 for ELISA, 10 for PRNT) for the purpose of calculations.

Descriptive statistics will be derived for Visits 1 and 4 including number of observations, geometric mean, and 95% CI (derived based on the antilogarithm of the 95% CI of the $\log_{10}$ titer transformations constructed using the t-distribution). GMTs and upper and lower confidence limits will be displayed to one decimal place.

The ratio of the GMTs will be presented between Groups 1-3 (Group 1/Group 2, Group 1/Group 3, and Group 2/Group 3) along with the 95% CI. For the ELISA the ratios of GMTs will be calculated for descriptive purposes.

GMTs will be graphically displayed using box and whisker plots for each group by visit (using the nominal week of the visit on the x-axis and a $\log_{10}$ scale for the y-axis).

Proportion of Results equal to or above LLOQ

Proportion of results equal to or above LLOQ will be presented for each visit along with the Clopper-Pearson 95% CIs.

Seroconversion rates

Seroconversion rate will be presented for Visit 4 along with the Clopper-Pearson 95% CIs of the seroconversion rate. In addition, the Freeman-Halton exact test will be performed to determine if there is any difference in seroconversion rates between the MVA-BN groups.

Correlation

Pearson's correlation coefficient (with associated 95% CI) between $\log_{10}$-transformed antibody titers measured by ELISA and PRNT at Visit 4 will be calculated along with the associated p-value for the PPS by group. These will also be presented on scatter plots.

Safety and Reactogenicity Endpoints

For each of the following endpoints, occurrence is defined as the frequency and incidence of subjects within a lot group experiencing the event. Event counts will also be summarized.

Serious AEs (SAEs) are those which meet one or more of the protocol-specified seriousness criteria:

- Results in death
- Is life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Results in a congenital anomaly or birth defect
- Otherwise important medical event

Causality is collected as none, unlikely, possibly, probably, or definitely related. AEs with a causality of possibly, probably, or definitely related, or those with missing causality assessments, will be considered related to vaccination for analysis purposes. AEs assessed as unlikely or not related will not be considered related to vaccination for the purpose of this trial.

Intensity for unsolicited AEs is graded using the *Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials*, published September 2007. Intensity grades are as follows: Grade 1/Mild, Grade 2/Moderate, Grade 3/Severe, Grade 4/Life-threatening, Grade 5/Fatal. Intensity for solicited AEs is defined in the corresponding section below.

AESIs include any cardiac sign or symptom developed since the first vaccination, ECG changes determined to be clinically significant, and post-vaccination cardiac enzyme Troponin I results above the upper limit of normal (Grade 1 or higher toxicity per the above-mentioned toxicity scale). In particular, clinically significant changes in ECG results and

elevated Troponin I results will be recorded as AEs and flagged as AESIs in the corresponding CRF. Data review of the ECG and Troponin I results versus the AE CRF page will be performed to ensure all applicable AESIs are captured.

Safety and Reactogenicity Variables

Unsolicited AEs

Occurrence, relationship and intensity of:

- SAEs
- Cardiac sign or symptom indicating a case of myo-/pericarditis (AESIs)
- Unsolicited AEs within 28 days after each vaccination

Occurrence of any related AEs and AEs ≥ Grade 3.

Solicited Local AEs

Solicited local AEs reported in the subject memory aid

- Injection Site Erythema
- Injection Site Swelling
- Injection Site Pain
- Injection Site Induration
- Injection Site Pruritus

Occurrence, intensity and duration of solicited local AEs during the 8-day period after each vaccination. Note, solicited local AEs are always assumed to be related to vaccination.

Injection site erythema, swelling and induration will be measured using a provided ruler and the maximum diameter will be recorded for each day on the memory aid. The intensity for these symptoms will be graded as follows:

Symptom	Grade	Intensity
Injection site erythema, swelling, and induration	0	0
	1	< 30 mm
	2	≥ 30 – < 100 mm
	3	≥ 100 mm
Injection site pruritus	0	No symptoms
	1	Mild - routine daily activities not impaired
	2	Moderate - routine daily activities impaired
	3	Severe - prevents routine daily activities
Injection site pain	0	No pain

	1	Painful on touch
	2	Painful when moving the limb
	3	Spontaneously painful / prevents normal activity

Solicited General AEs

Solicited general AEs reported in the subject memory aid

- Body Temperature (Increased to ≥ 99.5° F)
- Headache
- Myalgia
- Chills
- Nausea
- Fatigue

Occurrence, relationship, intensity and duration of solicited general AEs during the 8-day period after each vaccination.

Intensity of solicited general AEs is graded based on the memory aid data as follows:

Symptom	Grade	Intensity
Body temperature*	0	< 99.5°F (< 37.5°C)
	1	≥ 99.5 – < 100.4°F (≥ 37.5 – < 38.0°C)
	2	≥ 100.4 – < 102.2°F (≥ 38.0 – < 39.0°C)
	3	≥ 102.2 – < 104°F (≥ 39.0 – < 40.0°C)
	4	≥ 104°F (≥ 40.0°C)
Headache, Myalgia, Nausea, Chills and Fatigue	0	No symptoms
	1	Mild: routine daily activities not impaired
	2	Moderate: routine daily activities impaired
	3	Severe: prevents routine daily activities

*Pyrexia is defined as oral temperature ≥ 100.4°F (≥ 38.0 C).

Relationship will be determined by the investigator upon review of the memory aid at the visit following the vaccination.

Analysis

An overall summary of solicited and unsolicited AEs by vaccination period (Vaccination Period 1, Vaccination Period 2, Overall Vaccination Period, FU Period), including subject and event counts will be created with the following event categories:

- All AEs
- Related AEs*

- SAEs
- Related SAEs
- AESIs
- Related AESIs
- AEs ≥ Grade 3
- Related AEs ≥ Grade 3
- AEs Leading to Withdrawal from Second Vaccination*
- AEs Leading to Withdrawal from Trial*
- Fatal AEs

Note, for the FU period only SAEs and AESIs are expected to be reported, so the categories with the "" will not be reported for the FU Period. In addition, no solicited AEs categories will be reported during the FU Period.

Summary tables for unsolicited AEs by SOC and PT will be presented including frequencies (subject and event counts) and incidences (of subjects only) by vaccination period (Vaccination Period 1, Vaccination Period 2, Overall Vaccination Period, and FU Period) of the following event categories:

- AEs
- Adverse Drug Reactions (ADRs; related AEs)
- AEs ≥ Grade 3
- ADRs (related AEs) ≥ Grade 3
- Non-serious AEs
- Non-serious AEs in > 5% of Subjects
- SAEs
- AESIs
- AEs Leading to Withdrawal prior to Second Vaccination
- AEs Leading to Withdrawal from Trial

Tables summarized by SOC and PT will be sorted in order of descending incidence of SOCs in the overall column, and descending order of incidence of PTs within the SOCs. For subject level frequencies and percentages, subjects experiencing an event more than once will be counted only once within SOC and PT, however all events will be counted in the event column.

All AEs will be listed by lot group, subject, onset date, System Organ Class (SOC), and Preferred Term (PT). Any new unsolicited AE, with onset later than 35 days since the last vaccination or Visit 5, whichever is later, will not be included in summary tables but will be included in subject level listings.

Separate listings will be created for SAEs, AESIs, AEs leading to withdrawal from second vaccination, and AEs leading to withdrawal from the trial.

Solicited Local AEs

Note, only solicited local memory aid events with intensity grades above 0 are considered AEs. Solicited local AEs will be summarized by local AE term after each vaccination and overall by maximum intensity using subject frequencies and incidences. In addition, duration of local AEs will be summarized using n, mean, SD, median, minimum, and maximum.

The solicited local AE information including duration of the AEs will be included in a listing.

Solicited General AEs

Note, only solicited general memory aid events with intensity grades above 0 are considered AEs. Solicited general AEs will be summarized and listed in the same manner as solicited local AEs.

Additional summaries of the subset of general AEs determined to be related to vaccination, as well as those considered both related and ≥ Grade 3, will also be presented by subject frequencies and percentages.

4.4.6 Disposition and Trial Population Information

4.4.6.1 Disposition

All subjects screened will be accounted for in disposition summaries. A summary table will be presented specifying the number of subjects screened, randomized, treated/vaccinated, receiving both vaccinations, completing the active phase of the trial, included in each analysis set, withdrawing prior to the second vaccination (and reason), withdrawing from the trial (and reason), completing the FU visit overall, and FU visits by phone or on-site.

A listing will present all randomized subjects, date of completion/discontinuation, date of last vaccination, and reason for withdrawal from second vaccination or trial. All subjects not eligible for the trial will be listed including the reason for ineligibility.

4.4.6.2 Analysis Populations

Frequencies and percentages of each analysis population will be presented by lot group and overall. Reasons for exclusion from any analysis population will be included in the subject-level listings.

4.4.6.3 Protocol Deviations

Protocol deviations are collected on both a site and subject level basis. Subject level deviations will be databased and listed. Categorized deviations will be presented using frequencies and percentages in a table for the FAS.

4.4.6.4 Demographics and Baseline Characteristics

Demographic Variables

- Age at Informed Consent [years]
- Age Group (18 - median, > median - 45)
- Sex (Male, Female)
- Race (White, Black or African American, Asian, Native Hawaiian or Other Pacific Islander, American Indian or Alaska Native, Other)
- Race Group (White, Other)
- Ethnicity (Hispanic or Latino, Not Hispanic or Latino)
- Height [cm]
- Body weight [kg]
- BMI [kg/m^2]

Analyses

Listings will be presented for all data in the database. Tables of descriptive statistics for demographics will be produced for the FAS and PPS, individually. Descriptive statistics will be presented for the continuous demographic variables. Categorical demographic and baseline variables will be summarized using frequencies and percentages.

4.4.6.5 Medical History and Baseline Signs and Symptoms

Medical history data are collected at screening and include conditions that started prior to the signing of the ICF. These data are coded to MedDRA dictionary SOCs and PTs. Summaries of medical history events will be created by SOC and PT for the FAS. SOCs and PTs within SOCs will be sorted by descending frequency in the overall column.

Baseline signs and symptoms are defined as AEs that occur between the signing of the ICF and the first vaccination. These events are reported along with the AEs in the eCRF, but will be summarized separately from AEs. Like medical history events, they will be coded to MedDRA SOCs and PTs. Baseline signs and symptoms will be listed by SOC and PT within each subject. Relative day of onset will be calculated as described in Section 4.3.3.

4.4.6.6 Prior and Concomitant Medication

All prior and concomitant medication will be coded to WHO Drug Dictionary Global B3 version 2019 March ATC classes and preferred names. Displays of **prior** medications include medications where end date is before date of first administration of trial vaccination. Displays of **concomitant** medications include all ongoing medications, medications with missing end dates, or medications with end date after the first administration of trial vaccination. Tables by ATC Level 2 class and preferred name will be presented for the FAS. If ATC Level 2 class is not available, the next highest class available will be used. Subject level listings will be created based on the original eCRF data.

4.4.6.7 Non-drug Therapies and Procedures

All non-drug therapies and procedures will be listed by lot group, subject, SOC and PT. No summary tables will be created.

4.4.6.8 Exposure

Variables

- Number and percentage of subjects receiving 1 and 2 vaccinations
- Number of subjects returning memory aids

Analyses

Exposure to trial vaccine will be summarized by the number of vaccinations received as well as the number and percentage of subjects returning memory aids by vaccination in the FAS. All exposure data will be listed.

4.4.7 Safety Variables and Analyses

4.4.7.1 Adverse Events

See Section 4.4.5.2.

4.4.7.2 Clinical Laboratory Assessments

Performed at Screening, Visit 2, Visit 4, and FU if clinically indicated.

Variables

Serum chemistry test

- Total bilirubin
- Alkaline phosphatase
- AST
- ALT
- Serum creatinine
- Sodium
- Potassium
- Calcium
- Troponin I (mandatory only at screening)

Hematology

- RBC (erythrocyte) count
- Hemoglobin
- WBC (leukocyte) count and 5-part differential (Eosinophils, Basophils, Neutrophils, Lymphocytes, Monocytes)
- Platelet count

Pregnancy tests

Serum β-HCG pregnancy test at Screening and urine β-HCG pregnancy test within 24 hours prior to each vaccination and at last active trial visit (Visits 1, 3, and 5) are required for women of childbearing potential. Information on all pregnancy tests will be listed.

Analyses

For the purpose of analysis, laboratory data will be converted to standard Système International d'Unités (SI) units during creation of the Study Data Tabulation Model (SDTM) datasets. The original laboratory values and units will also be stored in the SDTM datasets. Only the SI units will be used in tables and listings. SI units and conversions will be included in the SDTM documentation.

All measured laboratory values will be listed and summarized at each scheduled visit using descriptive statistics. Out of range laboratory values will be flagged as either "L" for below normal range or "H" for above normal range in listings. Clinically significant abnormal laboratory values will be listed separately as part of the ICH reporting requirements.

Toxicity will be graded based on the *Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials*, published September 2007 (see Clinical Trial Protocol Appendix 1).

Summary tables will be produced for the number of high and low laboratory values at each visit by laboratory category and parameter. Pregnancy test results will be included only in listings.

"Shift tables" will be used to evaluate categorical changes in toxicity levels from baseline to Visit 2, Visit 4, and Worst Toxicity in the Overall Vaccination Period, respectively, for laboratory parameters graded per the above toxicity scale. For laboratory parameters not graded per the scale, similar shift tables will be created instead based on normal ranges (Low, Normal, High).

The frequency and proportion of subjects with a clinically significant laboratory result will be summarized by laboratory category and parameter for each post-vaccination visit as well as overall for the trial.

Any laboratory parameters which are not included in the protocol but reported by the central or local laboratories (e.g., in order to define an AE) will be listed but not tabulated.

4.4.7.3 Vital Signs

At each in-clinic visit, and FU if clinically indicated

Variables

- Heart rate [beats per minute]
- Systolic and diastolic blood pressure [mmHg]
- Body temperature [°C]

Analyses

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

4.4.7.4 ECGs

Mandatory at Screening, if clinically indicated at Visit 2 and Visit 4.

Variables

- Investigator's overall interpretation
- Investigator-rated clinical significance
- Central ECG reviewer results

- Heart Rate [bpm]
- RR [msec]
- PR interval [msec]
- QRS interval [msec]
- QRS Axis [deg]
- QT Interval [msec]
- QTcB (Bazett's Correction) Interval [msec]
- QTcF (Fridericia's Correction) Interval [msec]

Analyses

All ECG results will be listed by lot group, subject, and visit. As post-vaccination ECGs are only performed if clinically indicated, no summary tables will be created.

A listing of subjects with post-vaccination ECGs determined to be clinically significant will be produced.

4.4.7.5 Physical examination

The performance of physical examinations will be listed. Findings upon physical examination will be added to the Medical History CRF page if they started prior to the signing of the ICF, and to the AEs CRF page if starting after signing of the ICF. Findings occurring after the signing of the ICF, but pre-vaccination will be categorized as baseline signs and symptoms, and post-vaccination events will be considered part of the overall vaccination period.

4.4.8 Examination of Subgroups

Subgroup analyses by clinical trial site, sex (male vs. female), ethnicity (Hispanic or Latino vs. Not Hispanic or Latino), race group (White vs. Other), and age group (18 - median vs. > median - 45 years) will be performed for the primary immunogenicity analysis, overall summary of AEs, unsolicited AEs by SOC and PT, Solicited Local AEs by Maximum Intensity, and Solicited General AEs by Maximum Intensity.

4.5 Alterations to the Clinical Trial Protocol

No changes from the protocol specified analyses are planned.

5 References

1. International Conference on Harmonisation (July 1996). E3 Structure and Content of Clinical Study Reports.
2. International Conference on Harmonisation (September 1998). E9 Statistical Principals for Clinical Trials.
3. Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/toxicity-grading-scale-healthy-adult-and-adolescent-volunteers-enrolled-preventive-vaccine-clinical.
4. Ganju, J., Izu, A., Anemona, A (2008). Sample size for equivalence trials: A case study from a vaccine lot consistency trial. Statist. Med, 3743 – 3754.

Appendix 1: Sample Code for Multiple Imputation Analysis

The following is sample code for the MI sensitivity analysis of the primary endpoint.
TITERS: the dataset containing the PRNT titer data along with the covariates used in the analysis. Note, the baseline and post-baseline PRNT values need to be on the same record for the PROC MI procedure.
SEX: The gender identifier
YOB: Year of birth
RACE: The race identifier
LPRNTBL: Log_{10} Baseline PRNT value
LPRNTPB: Log_{10} Post-baseline PRNT value

```
proc mi data=titersl out=titermi nimpute=100 seed=31031 minimum=1;
    class sex race;
    var  yob sex race lprntbl lprntpb;
    fcs nbiter=20 regpmm(lprntbl=yob sex race/details );
    fcs nbiter=20 reg(lprntpb= lprntbl yob sex race /details);
run;
proc means data=titermi noprint nway;
 by _imputation_;
 class trtp;
 var  lprntpb;
 output out=summ(drop=_type_ _freq_) n=n mean=logmn stderr=logse ;
run;
proc sort data=summ;
    by trtp _imputation_;
run;

ods output parameterestimates=groupest;
proc mianalyze data=summ ;
    by trtp;
    modeleffects logmn;
    stderr logse;
run;
data groupest2;
    set groupest;
    GMT=10**estimate;
    GMT_LCL=10**LCLmean;
    GMT_UCL=10**UCLMean;
    keep trtp estimate lclmean uclmean gmt:;
    label estimate='Mean Log10 Titers'
          lclmean='Lower 95% CI of the Mean Log10 Titers'
          uclmean='Upper 95% CI of the Mean Log10 Titers'
          gmt='Geometric Mean Titer'
          GMT_LCL='Lower 95% CI of the Geometric Mean Titer'
          GMT_UCL='Upper 95% CI of the Geometric Mean Titer'
          Trtp='Treatment Arm';
run;

%macro difference(treats,dsetname);
ods select none;
```

```
ods output statistics=diff&dsetname.;
proc ttest data=titermi;
    by  imputation ;
    where trtp in (&treats.);
    class trtp;
    var lprntpb;
run;
ods output close;
ods select all;

ods output parameterestimates=diff&dsetname.c;
proc mianalyze data=diff&dsetname. (where=(class="Diff (1-2)"));
    modeleffects mean;
    stderr stderr;
run;
ods output close;

%mend;
%difference(%nrstr('Group 1' 'Group 2'),12);
%difference(%nrstr('Group 1' 'Group 3'),13);
%difference(%nrstr('Group 2' 'Group 3'),23);

data diffs;
    set diff12c (in=a) diff13c (in=b) diff23c (in=c);
    if a then label='Group 1 vs. Group 2';
    if b then label='Group 1 vs. Group 3';
    if c then label='Group 2 vs. Group 3';
    GMT Ratio=10**estimate;
    GMT Ratio LCL=10**lclmean;
    GMT_Ratio_UCL=10**uclmean;

    label estimate='Difference in Log10 Mean Titers'
          lclmean='Lower 95% CI for Difference in Log10 Mean Titers'
          uclmean='Upper 95% CI for Difference in Log10 Mean Titers'
          GMT Ratio='Geometric Mean Titer Ratio'
          GMT_Ratio_LCL='Lower 95% CI for Geometric Mean Titer Ratio'
          GMT_Ratio_UCL='Upper 95% CI for Geometric Mean Titer Ratio'
          label='Comparison Performed'
          ;
    keep label gmt: estimate lclmean uclmean;
run;
```