Crucell Holland BV

Clinical Protocol

A Phase 1, First-in-Human Study to Evaluate the Safety, Tolerability and Immunogenicity of Heterologous Prime-Boost Regimens Using MVA-BN®-Filo and Ad26.ZEBOV Administered in Different Sequences and Schedules in Healthy Adults

Protocol VAC52150EBL1001 Amendment 5; Phase 1

VAC52150 (Ad26.ZEBOV/MVA-BN-Filo [MVA-mBN226B])

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Protocol History VAC52150EBL1001_Protocol			
Document Type and *File Name*	Issued Date	Amendment Type	Comments
Initial Clinical Protocol *VAC52150EBL1001_Protocol*	27-Nov-2014	-	-
Protocol Amendment 1 *VAC52150EBL1001_Protocol_Amend_1*	8-Dec-2014	Substantial	For details, see Section Amendment_1
Protocol Amendment 2 *VAC52150EBL1001_Protocol_Amend_2*	18-Dec-2014	Substantial	For details, see Section Amendment_2
Protocol Amendment 3 *VAC52150EBL1001_Protocol__Amend_3*	12-Jan-2015	Substantial	For details, see Section Amendment_3
Protocol Amendment 4 *VAC52150EBL1001_Protocol__Amend_4*	21-Jan-2015	Substantial	For details, see Section Amendment_4
Protocol Amendment 5 *VAC52150EBL1001_Protocol__Amend_5*	This document	Substantial	For details, see Section Amendment_5

TABLE OF CONTENTS

LIST OF ATTACHMENTS

LIST OF IN-TEXT TABLES

TABLES

PROTOCOL AMENDMENT

Amendment_5 (this document)

The overall reason for the amendment: This amendment was written to add additional interim analyses of immunogenicity data and the use of blood samples for future scientific research. Additional changes were made to the visit window of the 21-day post-boost visit, to clarify that all subjects will be contacted 24 hours after each vaccination in order to collect adverse events, and to clarify that the sponsor will be unblinded for the 7-day post-prime interim analysis. Further modifications were made throughout the protocol to correct minor inconsistencies and for clarity.

The changes made to the clinical protocol VAC52150EBL1001 Amendment 4, dd. 21-Jan-2015, are listed below, including the rationale of each change and a list of all applicable sections.

Rationale: For the purpose of informing future vaccine-related decisions in a timely manner, the possibility of conducting additional interim analyses was added.

SYNOPSIS
11 STATISTICAL METHODS
11.6 Interim Analyses
11.7 Data Review Committee (DRC)

Rationale: Wording was added to clarify that all subjects will be contacted 24 hours after each vaccination in order to collect adverse events.

SYNOPSIS
Time and Events Schedule
3.1 Overview of Study Design
9.1.4 Vaccination

Rationale: Wording was included to clarify that blood from immunologic samples may be used for future scientific research. No additional samples will be collected.

SYNOPSIS
9.2.2 Evaluations

Rationale: The sponsor personnel involved in the 7 days post-prime interim analysis will be unblinded at the time of the analysis.

5 TREATMENT ALLOCATION AND BLINDING

Rationale: The visit window for the 21-day post-boost visit was changed from 2 to 3 days.

9.1.2 Visit Windows

Amendment_4 (21-Jan-2015)

The overall reason for the amendment: This amendment was written to enroll an additional group of approximately 15 subjects (Group 5) to investigate a regimen using Ad26.ZEBOV as prime and MVA-BN-Filo as boost at a 14-day interval. It is believed that the 56-day interval may provide a more durable immune response than the shorter intervals, however, the shorter intervals are more optimal for use in an acute outbreak setting. Therefore, a need exists to study the 14-day interval next to the 28- and 56-day interval. The 14-day interval regimen that will be explored in Group 5 may be further evaluated in future studies. The design of this substudy will be open-label, uncontrolled and non-randomized. All other key elements of the substudy, including entry

criteria and safety and immunogenicity evaluations, will be similar to the main study (Groups 1 to 4).

Furthermore, additional exploratory immunologic assays are added to the protocol. Intracellular cytokine staining (ICS) and ELISpot analysis of frozen peripheral blood mononuclear cells (PBMC) was included in the original protocol. These assays will be performed in all studies with VAC52150 to assure cross-comparability. However, due to the freezing procedure CD4-positive and low-magnitude T-cell responses are diminished. To be able to detect the whole range of responses in subjects of this study, the performance of ICS and/or ELIspot of fresh PBMC is now added (depending on assay and sample availability at relevant study time points). An exploratory ELISA is introduced that provides additional insight into the binding antibody response using a different source of EBOV glycoprotein (GP) and read-out. Furthermore, results obtained with this assay have been validated using a set of therapeutic antibodies.

The changes made to the clinical protocol VAC52150EBL1001 Amendment 3, dd. 12-Jan-2015, are listed below, including the rationale of each change and a list of all applicable sections.

Rationale: The protocol was updated to add an open-label treatment arm to investigate a regimen using Ad26.ZEBOV as prime and MVA-BN-Filo at a 14-day interval.

SYNOPSIS
Time and Events Schedule
1 INTRODUCTION
3 STUDY DESIGN AND RATIONALE
4 SUBJECT POPULATION
5 TREATMENT ALLOCATION AND BLINDING
6 DOSAGE AND ADMINISTRATION
9 STUDY EVALUATIONS
10 SUBJECT COMPLETION/WITHDRAWAL
11 STATISTICAL METHODS
12 ADVERSE EVENT REPORTING
14 STUDY VACCINE INFORMATION
16 ETHICAL ASPECTS

Rationale: The protocol was updated to add additional exploratory immunologic assays.

SYNOPSIS
9.2.2 Evaluations

Amendment_3 (12-Jan-2015)

The overall reason for the amendment: This amendment was written to add an extra interim analysis, to clarify the data sets used for immunogenicity analyses, and to update the list of references.

The changes made to the clinical protocol VAC52150EBL1001 Amendment 2, dd. 18-Dec-2014, are listed below, including the rationale of each change and a list of all applicable sections.

Rationale: The protocol was updated to add an interim analysis for decision-making for future studies.

SYNOPSIS
5 TREATMENT ALLOCATION AND BLINDING
11 STATISTICAL METHODS

11.6 Interim Analyses

Rationale: The reference list was updated to include the addendum to the Ad26.Mos.HIV Investigator Brochure that was issued in December 2014.

1.1 Background
REFERENCES

Rationale: It was clarified that the Per Protocol (PP) analyses of immunogenicity will only be performed if more than 10% of subjects from the Immunogenicity Response (IR) analysis set are excluded from the PP analysis set.

11.4 Immunogenicity Analyses

Amendment_2 (18-Dec-2014)

The overall reason for the amendment: This amendment was written to clarify the birth control requirements for women of childbearing potential. Further, the details on Ad26.ZEBOV and MVA-BN-Filo preparation were removed from the protocol, and clarifications and corrections were made.

The changes made to the clinical protocol VAC52150EBL1001 Amendment 1, dd. 8-Dec-2014, are listed below, including the rationale of each change and a list of all applicable sections.

Rationale: It was clarified that unsolicited adverse events are also to be recorded by the subjects in the diary. This was inconsistent in the original protocol.

SYNOPSIS
Time and Events Schedule
1.2.5 Overall Benefit/Risk Assessment
3.1 Overview of Study Design

Rationale: The original protocol used incorrect wording for the EBOV GP in association with immunologic assays/responses. This was corrected in the amendment.

SYNOPSIS
2.1 Objectives
9.2.1 Endpoints
9.2.2 Evaluations

Rationale: The text in the original protocol could be interpreted as if subjects in both cohorts would be enrolled on the same day. This has been reworded in the amendment.

SYNOPSIS
3.1 Overview of Study Design

Rationale: In the original protocol, subjects who had received active vaccine were asked to consent for enrollment into the VAC52150 Vaccine Development Registry Protocol. This amendment clarifies that also subjects who have received placebo should be approached to consent for enrollment in the Registry.

SYNOPSIS
3.1 Overview of Study Design
9.1.7 VAC52150 Vaccine Development Registry

Rationale: In the original protocol, solicited systemic reactions and solicited systemic adverse events were used interchangeably. This has been made consistent in the amendment.

SYNOPSIS

3.1 Overview of Study Design

11.8 Pausing Rules

Rationale: In this amendment, text is added that for administering the vaccine, the study vaccine administrator should consider whether there is an injury, skin problem, or tattoo that precludes administering the injection or that will interfere with evaluating the arm after injection.

SYNOPSIS

6 DOSAGE AND ADMINISTRATION

Rationale: In the original protocol, the confidence limit associated with the true rate of having 0 adverse events that preclude further study vaccine administration or more serious events that would limit product development was wrong. This has been corrected in the amendment.

SYNOPSIS

11.2 Sample Size Determination

Rationale: In the original protocol, there was no text or footnote in the Time and Event Schedule for the collection of concomitant therapies during long-term follow-up. This has been added in the amendment.

Time and Events Schedule

Rationale: In the original protocol, there was an error in the number of subjects exposed to MVA-BN-based vaccines in completed and ongoing clinical studies. This has been corrected in the amendment.

1.1 Background

1.3 Overall Rationale for the Study

Rationale: In the original protocol it was stated that subjects had to use adequate birth control measures until at least 3 months after the boost vaccination. In the amendment it is clarified that subjects who have received placebo may stop birth control measures or resume their pre-study birth control measures after unblinding.

1.2.4 Potential Risks

4.3 Prohibitions and Restrictions

Rationale: In inclusion criterion #4 in the original protocol, it was erroneously stated that the values for serum creatinine, troponin I, fibrinogen and prothrombin time should be <1 x institutional upper limit of normal. This was corrected in the amendment to ≤1 x institutional upper limit of normal

4.1 Inclusion Criteria

Rationale: The original protocol had no wording around laboratory test results for parameters other than those mentioned in inclusion criterion #4. This has been added in the protocol amendment.

4.2 Exclusion Criteria

Rationale: The amendment includes clarification that for women of childbearing potential, it should be confirmed that adequate birth control measures were used from at least 28 days prior to study immunization with a negative serum β-hCG pregnancy test available before Day 1 and a negative urine test immediately prior to each study vaccination.

4.1 Inclusion Criteria

9.1.3 Screening

Rationale: In the subsection on Solicited Systemic Adverse Events, the sentence "*Fever will be recorded by the investigator or by his/her designee for temperatures equal to or higher than 38.0°C*" was removed from the protocol. During Day 1 to Day 8, fever will be derived from the temperature as recorded in the subject diary. Also if the temperature is above 38.0°C beyond Day 8 but starting at or before Day 8. So, no additional reporting of an adverse event (AE) is needed.

9.3.2 Evaluations

Rationale: In the original protocol, it was not clear how solicited adverse events that were ongoing at Day 8 would be captured. This has been clarified in the amendment.

9.3.2 Evaluations

Rationale: Subjects should be discontinued from the study if the randomization code is broken by the investigator or the study-site personnel. This was an inconsistency in the original protocol.

10.2 Discontinuation of Study Vaccine

Rationale: Text was added to clarify the replacement of subjects who prematurely withdraw from the study.

10.4 Withdrawal From the Study

Rationale: The definition of the Per Protocol Analysis Set was changed to clarify that vaccinated subjects should have received both the prime and boost vaccinations.

11.1 Analysis Sets

Rationale: In the original protocol, oral body temperature was erroneously left out from the description of descriptive statistics. This has been added in the amendment.

11.5 Safety Analyses

Rationale: In the original protocol, the manufacturer for the MVA-BN-Filo vaccine was wrong. This has been corrected in the amendment.

14.1 Description of Study Vaccines

Rationale: The details on Ad26.ZEBOV and MVA-BN-Filo preparation were removed from the protocol. They are available in the Site Investigational Product Procedures Manual.

14.3 Preparation, Handling and Storage

Rationale: Other minor clarifications and corrections were made throughout the protocol.

Amendment_1 (8-Dec-2014)

The overall reason for the amendment: This amendment was written in response to a Notice of Grounds for Non-Acceptance and Right to Amend Request from the health authorities, received on 3-Dec-2014, and in response to the Provisional Opinion of the Research Ethics Committee, received on 5-Dec-2014.

The changes made to the clinical protocol VAC52150EBL1001, dd. 27-Nov-2014, are listed below, including the rationale of each change and a list of all applicable sections.

Rationale: The amendment includes a definition of solicited adverse events in the context of vaccine clinical studies to distinguish from the definitions based on pharmacovigilance guidelines.

Definitions of Terms

Rationale: The amendment includes a rationale for Ad26 seropositivity assessment at baseline.

1.1 Background, Clinical Studies, Safety Profile of ad26.ZEBOV

Rationale: The original protocol erroneously stated that although routinely used by the subcutaneous route, MVA-BN-**Filo** at a dose of $1x10^8$ $TCID_{50}$ has been demonstrated to be as safe and immunogenic when used by the intramuscular route. In the amendment, this has been corrected to be […] **MVA-BN** at a dose of […].

1.3 Overall Rationale for the Study

Rationale: In the original protocol, the subjects in the Sentinel Cohorts and in Cohort 1 of each group were to be contacted by telephone in the morning after the study vaccination to verify the absence of any predefined adverse events. In the amendment, the follow-up telephone call for these cohorts has been changed to be after 24 hours instead of the following morning.

SYNOPSIS

3.1 Overview of Study Design

9.1.4 Vaccination

Rationale: The amendment includes a rationale for the selected doses of both study vaccines.

3.2 Study Design Rationale

Rationale: The amendment includes a clarification for the hemoglobin value for male subjects in the inclusion criteria.

4.1 Inclusion Criteria

Rationale: In the original protocol, there was an error in the amount of blood volume to be drawn. This has been corrected in the amendment.

Time and Events Schedule

9.1.1 Overview

16.1 Study-Specific Design Considerations

Rationale: In the original protocol, it was stated that the primary objective of the VAC52150 Vaccine Development Registry is to collect cumulative serious adverse event information and pregnancy outcomes from all subjects who have entered the first VAC52150 clinical development study (or another eligible study) up to 4 years after the end of the present study or until the product has been withdrawn from development. In the amendment, the last part ("or until the product has been withdrawn from development") has been removed.

9.1.7 VAC52150 Vaccine Development Registry

Rationale: In the amendment, a statement has been added to clarify that if any pausing rule is met, and following an internal safety review it is deemed appropriate to restart dosing, a request to restart dosing with pertinent data must be submitted to the health authorities as a request for a substantial amendment. In addition, the pausing rules for Cohort 2 have been updated.

11.8 Pausing Rules

Rationale: The amendment includes the collection of adverse events of interest as well as a statement to clarify that a troponin I increase will always be considered unexpected for regulatory purposes in the current study.

12.1.1 Adverse Event Definitions and Classifications

Rationale: Minor clarifications and corrections

Title page
1 INTRODUCTION
3.1 Overview of Study Design
5 TREATMENT ALLOCATION AND BLINDING
9.2.1 Endpoints
11.5 Safety Analyses
14.3 Preparation, Handling and Storage
REFERENCES

SYNOPSIS

A Phase 1, First-in-Human Study to Evaluate the Safety, Tolerability and Immunogenicity of Heterologous Prime-Boost Regimens Using MVA-BN®-Filo and Ad26.ZEBOV Administered in Different Sequences and Schedules in Healthy Adults

OVERALL RATIONALE

This first-in-human study consists of 2 parts. The first part, ie, the main study, is a randomized, placebo-controlled, observer-blind study, and will evaluate the Crucell adenovirus serotype 26 (Ad26) vector expressing the glycoprotein (GP) of the Ebola virus (EBOV) Mayinga variant (**Ad26.ZEBOV**) and the Modified Vaccinia Ankara (MVA) - Bavarian Nordic (BN) vector with EBOV, Sudan virus (SUDV) and Marburg virus (MARV) GP inserts and Tai Forest virus (TAFV) nucleoprotein (NP) insert (**MVA-BN-Filo**) as a heterologous prime-boost regimen, in which one study vaccine is used to prime a filovirus-specific immune response and the other study vaccine is used to boost the immune response 28 or 56 days later. The second part is an open-label, uncontrolled substudy that will evaluate a regimen in which Ad26.ZEBOV is used as a prime, and MVA-BN-Filo is given as a boost 14 days later. The EBOV GP that is currently circulating in West Africa has 97% homology to the EBOV GP used in this vaccine regimen.

OBJECTIVES AND HYPOTHESIS

This study aims to evaluate the safety, tolerability and immunogenicity of different MVA-BN-Filo and Ad26.ZEBOV vaccine regimens.

Primary Objectives

- To assess the safety and tolerability of heterologous prime-boost regimens utilizing MVA-BN-Filo at a dose of $1x10^8$ $TCID_{50}$ (50% Tissue Culture Infective Dose) and Ad26.ZEBOV at a dose of $5x10^{10}$ vp (viral particles).

Secondary Objectives

- To assess humoral and cellular immune responses to the EBOV GP of the various regimens tested as measured by virus neutralization assay, enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunospot (ELISpot) assay.

Exploratory Objectives

- To explore further humoral and cellular immune responses to the EBOV GP and the adenovirus and MVA backbone of the various regimens tested. The assays to be used may include, but will not be

limited to, adenovirus/MVA neutralization assays, molecular antibody characterization, and intracellular cytokine staining (ICS).

- To explore humoral and cellular immune responses to the SUDV GP, MARV GP and TAFV NP if assays are available.

Hypothesis

No formal statistical hypothesis testing is planned.

OVERVIEW OF STUDY DESIGN

This Phase 1 first-in-human study consists of 2 parts. The main study is a randomized, placebo-controlled, observer-blind study evaluating the safety, tolerability and immunogenicity of 4 regimens using MVA-BN-Filo at a dose of $1x10^8$ $TCID_{50}$ and Ad26.ZEBOV at a dose of $5x10^{10}$ vp: 2 regimens will have MVA-BN-Filo as prime and Ad26.ZEBOV as boost at a 28- or 56-day interval, and 2 regimens will have Ad26.ZEBOV as prime and MVA-BN-Filo as boost at a 28- or 56-day interval. The main study will be conducted in approximately 72 healthy adult subjects who never received an experimental Ebola candidate vaccine before and have no known exposure to or diagnosis of Ebola disease. The open-label, uncontrolled substudy will evaluate the safety, tolerability and immunogenicity of a regimen with Ad26.ZEBOV at a dose of $5x10^{10}$ vp as prime, and MVA-BN-Filo at a dose of $1x10^8$ $TCID_{50}$ as boost 14 days later, and will be conducted in approximately 15 healthy adult subjects.

The study consists of a screening period (of up to 28 days in the main study and up to 56 days in the substudy), a vaccination period in which subjects will be vaccinated at baseline (Day 1) followed by a boost on Day 15, 29 or 57, and a post-boost follow-up until all subjects have had their 21-day post-boost visit (Day 36, 50 or 78) or discontinued earlier. The main study will be unblinded when all subjects in the main study have had their 21-day post-boost visit or discontinued earlier. After unblinding, subjects who received placebo will be contacted to communicate that they have completed the study and do not need to contact the site any longer. Subjects who received active vaccine will enter a long-term follow-up, with visits on Days 180 (±15 days), 240 (±30 days) and 360 (±30 days) post-prime.

The Principal Investigator (who is blinded to treatment in the main study) will be responsible for the safety monitoring of the study. If at least one pre-specified pausing rule is met, study vaccinations will be paused and an internal Data Review Committee (DRC) meeting will be convened.

Subjects in the main study will be enrolled into 4 different groups, comprising 18 healthy subjects each. Overall, subjects will be randomized within group in a 5:1 ratio to receive either active vaccine or placebo (0.9% saline) through intramuscular (IM) injections (0.5 mL) as follows:

- MVA-BN-Filo ($1x10^8$ $TCID_{50}$) administered on Day 1, followed by a booster of Ad26.ZEBOV ($5x10^{10}$ vp) on Day 29 (Group 1) or Day 57 (Group 2), or
- Ad26.ZEBOV ($5x10^{10}$ vp) administered on Day 1, followed by a booster of MVA-BN-Filo ($1x10^8$ $TCID_{50}$) on Day 29 (Group 3) or Day 57 (Group 4).

Enrollment of subjects in Groups 1 and 3 will start with vaccination of 1/1 subjects (active vaccine/placebo; Sentinel Cohort) to assess the tolerability of the 2 study vaccines over a 24-hour period before exposing larger cohorts of subjects to the vaccines. After at least 24 hours after the prime vaccination, subjects will be contacted by telephone to verify the absence of any predefined events (ie, a serious adverse event considered to be related to any of the study vaccines, signs of anaphylaxis or generalized urticaria clearly attributable to study vaccination, a severe [grade 3] unsolicited adverse event considered to be related to any of the study vaccines, a severe [grade 3] solicited injection site reaction, a severe [grade 3] solicited systemic adverse event considered to be related to any of the study vaccines, or death). Enrollment of the next cohorts will be as follows:

- Groups 1 and 3: 4/1 subjects (active vaccine/placebo; Cohort 1) followed, in the absence of any of the above-mentioned events, confirmed by a telephone call with each subject after at least 24 hours after the prime vaccination, by 10/1 subjects (active vaccine/placebo; Cohort 2)
- Groups 2 and 4: 5/1 subjects (active vaccine/placebo; Cohort 1) followed, in the absence of any of the above-mentioned events, confirmed by a telephone call with each subject after at least 24 hours after the prime vaccination, by 10/2 subjects (active vaccine/placebo; Cohort 2)

The enrollment of all subjects of Cohort 1 will be carried out ideally on the same day. Also the enrollment of all subjects of Cohort 2 will be carried out ideally on the same day. If the enrollment of Cohort 1 is carried out over more than 1 day, enrollment of Cohort 2 in that group will only start after at least 24 hours after the prime vaccination in the last subject in Cohort 1.

Enrollment in the open-label substudy (Cohort 3) will start after the prime vaccination in the last subject in Cohort 2. Subjects will receive active vaccine through IM injections (0.5 mL) as follows:

- Ad26.ZEBOV ($5x10^{10}$ vp) on Day 1, followed by a booster of MVA-BN-Filo ($1x10^{8}$ $TCID_{50}$) on Day 15 (Group 5).

The study enrollment plan is summarized in Table 1. The different study vaccination schedules are shown in Table 2.

Table 1: Study Enrollment Plan

Group	Sentinel Cohort	Cohort 1 [a)]	Cohort 2 [b)]	Cohort 3 [c)]
1	1:1 (active vaccine/placebo)	4:1 (active vaccine/placebo)	10:1 (active vaccine/placebo)	
2	-	5:1 (active vaccine/placebo)	10:2 (active vaccine/placebo)	
3	1:1 (active vaccine/placebo)	4:1 (active vaccine/placebo)	10:1 (active vaccine/placebo)	
4	-	5:1 (active vaccine/placebo)	10:2 (active vaccine/placebo)	
5	-	-	-	15 (active vaccine)

a) Cohort 1 will be enrolled after at least 24 hours after the prime vaccination in the Sentinel Cohorts.
b) Cohort 2 will be enrolled after at least 24 hours after the prime vaccination in the last subject in Cohort 1.
c) Cohort 3 will be enrolled after the prime vaccination in the last subject in Cohort 2 for operational/logistic reasons.

Table 2: Study Vaccination Schedules

Group	N		Day 1	Day 15	Day 29	Day 57
1	18	15	MVA-BN-Filo $1x10^{8}$ $TCID_{50}$	-	Ad26.ZEBOV $5x10^{10}$ vp	-
		3	placebo (0.9% saline)	-	placebo (0.9% saline)	-
2	18	15	MVA-BN-Filo $1x10^{8}$ $TCID_{50}$	-	-	Ad26.ZEBOV $5x10^{10}$ vp
		3	placebo (0.9% saline)	-	-	placebo (0.9% saline)
3	18	15	Ad26.ZEBOV $5x10^{10}$ vp	-	MVA-BN-Filo $1x10^{8}$ $TCID_{50}$	-
		3	placebo (0.9% saline)	-	placebo (0.9% saline)	-
4	18	15	Ad26.ZEBOV $5x10^{10}$ vp	-	-	MVA-BN-Filo $1x10^{8}$ $TCID_{50}$
		3	placebo (0.9% saline)	-	-	placebo (0.9% saline)
5	15		Ad26.ZEBOV $5x10^{10}$ vp	MVA-BN-Filo $1x10^{8}$ $TCID_{50}$	-	-

N: number of subjects to receive study vaccine; $TCID_{50}$: 50% Tissue Culture Infective Dose; vp: viral particles

After each vaccination, subjects will be observed directly for 30 minutes and remain at the site for a total of 60 minutes post-vaccination to monitor for the development of any acute reactions, or longer in case of grade 3 adverse events. Subjects will be instructed to contact the investigator immediately if they experience any adverse event that they perceive as relevant or possibly related to study vaccine in their

opinion, and will be contacted after at least 24 hours for collection of adverse events and to confirm that no pre-specified pausing rules have been met. Subjects will use a diary to document symptoms of unsolicited and solicited local and systemic adverse events in the evening after each vaccination and then daily for the next 7 days. The investigator or the designee will document unsolicited adverse events from signing of the informed consent form (ICF) onwards until 21 days post-boost, and serious adverse events and adverse events related to blood draws from signing of the ICF onwards until the end of the study. In addition, the investigator or designee will collect samples for safety assessments (see Safety Evaluations below) and assessments of immune responses (see Immunogenicity Evaluations below) at the time points indicated in the Time and Events Schedule.

All subjects will be approached to consent for enrollment into the VAC52150 Vaccine Development Registry Protocol for long-term surveillance after completing the present study.

SUBJECT POPULATION

Screening for eligible subjects must be performed within 28 days (main study) or 56 days (substudy) before Day 1. Subjects must be healthy (on the basis of physical examination, medical history and clinical judgment) men and women, aged ≥18 to ≤50 years.

DOSAGE AND ADMINISTRATION

MVA-BN-Filo and Ad26.ZEBOV, or placebo, will be administered as IM injections in either deltoid in the upper arm. When choosing an arm for the injection, the study vaccine administrator should consider whether there is an arm injury, local skin problem, or significant tattoo that precludes administering the injection or will interfere with evaluating the arm after injection. The boost vaccination should be administered in the opposite deltoid from the prime vaccination.

Subjects in the main study will receive a vaccination, according to randomization, on Days 1 and 29 (Groups 1 and 3) or on Days 1 and 57 (Groups 2 and 4) at the following dose levels:

- MVA-BN-Filo added to a diluent consisting of tris buffered saline: $1x10^8$ $TCID_{50}$, 0.5 mL
- Ad26.ZEBOV: $5x10^{10}$ vp, 0.5 mL
- Placebo: 0.9% saline, 0.5 mL

Subjects in the substudy (Group 5) will receive active vaccine on Days 1 and 15 at the same dose levels as in the main study.

SAFETY EVALUATIONS

Safety will be assessed by collection of solicited local and systemic adverse events, unsolicited adverse events and serious adverse events as described above, and by physical examination. In addition, standard chemistry, hematologic (including coagulation parameters) and urinalysis parameters will be assessed at the time points indicated in the Time and Events Schedule. Vital signs (blood pressure, heart rate, oral body temperature) will be assessed at screening and before and at 30 and 60 minutes after each study vaccination. A single, 12-lead electrocardiogram (ECG) will be performed at screening and at 3 days after each study vaccination and may be repeated at other time points during the study if deemed necessary by the investigator.

Study vaccination will be paused if any of the pre-specified pausing rules is met.

IMMUNOGENICITY EVALUATIONS

Immunologic assays and their purposes are summarized in Table 3 and Table 4. The exploratory assay package may include, but will not be limited to, the listed assays. Sample collection and processing will be performed by the site staff according to current versions of approved standard operating procedures.

Table 3: Summary of Immunologic Assays (Serology)

Assay	Purpose
Secondary endpoints	
Virus neutralization assay	Analysis of neutralizing antibodies to EBOV GP
ELISA	Analysis of antibodies binding to EBOV GP
Exploratory endpoints	
Adenovirus/MVA neutralization assay	Neutralizing antibodies to adenovirus/MVA
Molecular antibody characterization	Analysis of anti-EBOV GP, SUDV GP, MARV GP and/or TAFV NP antibody characteristics, including IgG subtyping
Exploratory ELISA	Analysis of binding antibodies to a different source of EBOV GP

EBOV: Ebola virus; ELISA: enzyme-linked immunosorbent assay; GP: glycoprotein; IgG: immunoglobulin G; MARV: Marburg virus; MVA: Modified Vaccinia Ankara; NP: nucleoprotein; SUDV: Sudan virus; TAFV: Tai Forest virus

Table 4: Summary of Immunologic Assays (Cellular)

Assay	Purpose
Secondary endpoints	
ELISpot	T-cell IFN-γ responses to EBOV GP
Exploratory endpoints	
ICS of frozen PBMC	Analysis of T-cell responses to EBOV GP, SUDV GP, MARV GP and/or TAFV NP (including CD4/8, IL-2, IFN-γ, TNF-α and/or activation markers)
ICS and/or ELISpot of fresh PBMC	Analysis of T cell responses to EBOV GP including CD4-positive and low-magnitude T cell responses

EBOV: Ebola virus; ELISpot: enzyme-linked immunospot; GP: glycoprotein; ICS: intracellular cytokine staining; IFN: interferon; IL: interleukin; MARV: Marburg virus; NP: nucleoprotein; PBMC: peripheral blood mononuclear cells; SUDV: Sudan virus; TAFV: Tai Forest virus; TNF: tumor necrosis factor

Future scientific research may be conducted to further investigate Ebola vaccine- and disease-related questions. This may include the development of new or the improvement of existing techniques to characterize EBOV-directed immune responses or diagnostic tests. No additional samples will be taken for these analyses, however residual samples from other tests (eg, plasma from safety specimens) may be retained for these purposes.

STATISTICAL METHODS

Three interim analyses are planned in the main study for decision-making for future studies. The first interim analysis will assess safety data and will be performed when all subjects in the main study have completed the 7 days post-prime visit. The second interim analysis will be performed when all subjects in the Sentinel Cohorts and in Cohort 1 (26 subjects) have completed the 28 days post-prime visit to assess immunogenicity. The third interim analysis will be performed when all remaining subjects in Cohort 2 (46 subjects) have completed the 28 days post-prime visit to assess safety and immunogenicity. This analysis will include all available data from the main study up to this point.

Two interim analyses are planned in the substudy. The first interim analysis will assess safety data and will be performed when all subjects in Group 5 have completed the 7 days post-prime visit. The second interim analysis will be performed when all subjects have completed the 21-day post-boost visit (Day 36) or discontinued earlier. This analysis will include all available data from Group 5 up to this point.

Additional interim analyses may be performed during the study for the purpose of informing future vaccine-related decisions in a timely manner. The results will not influence the conduct of the study in terms of early termination or later safety or immunogenicity endpoint assessments.

The primary analysis will be performed on the main study only and will be done when all subjects in Groups 1 to 4 have completed the 21-day post-boost visit (Day 50 for Groups 1 and 3, and Day 78 for Groups 2 and 4) or discontinued earlier. This analysis will include all available data from the main study up to this point.

The final analysis will be performed when all subjects from Groups 1 to 5 have completed the last study-related visit or discontinued earlier.

In each group (Groups 1 to 5), 15 subjects will receive the active vaccine. Three subjects in each group in the main study (Groups 1 to 4) will receive placebo. The primary objective of this study is safety and tolerability.

The sample size is not based on formal hypothesis testing considerations, but is within the range of subjects as recommended by the International Conference on Harmonisation (ICH) for first-in-human products in this investigation. Placebo recipients are included in the main study for blinding purposes and safety analyses, and will provide control specimens for immunologic assays.

The sample size for this study will provide a preliminary safety and immunogenicity assessment. While mild to moderate vaccine reactions (local/systemic responses) are expected, adverse events that preclude further study vaccine administration or more serious events that would limit product development are not anticipated. With 15 subjects in the active vaccine group, the observation of 0 such reactions would be associated with a one-sided 97.5% confidence upper limit that the true rate is less than 22%. Table 5 provides the probabilities of observing at least one adverse event at given true adverse event rates.

Table 5: Probability of Observing at Least One Adverse Event Given a True Adverse Event Incidence

	Probability of Observing at Least One Adverse Event
True Adverse Event Incidence	**N=15**
1%	14%
2.5%	32%
5%	54%
10%	79%
20%	96%

N: number of subjects receiving active vaccine

TIME AND EVENTS SCHEDULE

(see the next pages)

TIME AND EVENTS SCHEDULE

	Screening (≤28 or 56 days)	Study Period							Long-term Follow-up [p]		
		Day 1									
Study Procedures											
Screening/Administrative [a]											
Informed consent [b]	X										
Inclusion/exclusion criteria	X	X [c]									
Medical history and demographics	X										
Prestudy therapies [d]	X										
Serum pregnancy test [e]	X										
Serology (HIV-1/2, hepatitis B/C)	X										
Follicle-stimulating hormone (FSH) [f]	X										
Urine drug screen	X										
Randomization (only for subjects in the main study)		X									

Groups 1 and 3		**Day 1**	**Day 4**	**Day 8**	**Day 29**	**Day 32**	**Day 36**	**Day 50 [q]**	**Day 180**	**Day 240**	**Day 360**
Study vaccine administration		▲[g]			▼[g]						
Safety Assessments											
Urine pregnancy test [e]		X [h]			X [h]						
Physical examination [j]	X	X			X			X			
Vital signs [i]	X	X [i]			X [i]						
12-Lead electrocardiogram (ECG) [k]	X		X			X					
Distribution of subject diary [l]		X			X						
Review of subject diary by site staff			X	X		X	X				
Adverse events	Continuous										
Serious adverse events	Continuous										
Adverse events related to blood draws	Continuous										
Concomitant medications		X	X	X	X	X	X	X	X [m]		
Clinical Laboratory Samples											
Chemistry, hematology [m] (mL)	X [o] (20)	X [h] (10)	X (10)	X (10)	X [h] (10)	X (10)	X (10)				
Urinalysis	X [o]	X [h]		X	X [h]		X				
Immunogenicity Blood Samples											
Peripheral blood mononuclear cell (PBMC) assay (mL)		X [h] (60)		X (40)	X [h] (40)		X (40)	X (100)	X (40)	X (40)	X (40)
Humoral assays (mL)		X [h] (25)		X (20)	X [h] (20)		X (20)	X (60)	X (20)	X (20)	X (20)
Approximate Blood Volumes											
Daily blood draws (mL)	20	95	10	70	70	10	70	160	60	60	60
Cumulative blood draws (mL)	20	115	125	195	265	275	345	505	565	625	685

Group 1: ▲MVA-BN-Filo 1x10^8 TCID$_{50}$ or placebo, ▼Ad26.ZEBOV 5x10^{10} vp or placebo Group 3: ▲ Ad26.ZEBOV 5x10^{10} vp or placebo, ▼ MVA-BN-Filo 1x10^8 TCID$_{50}$ or placebo

Other footnotes are provided after the Time and Events Schedule for Group 5.

Groups 2 and 4		**Day 1**	**Day 4**	**Day 8**	**Day 29**	**Day 57**	**Day 60**	**Day 64**	**Day 78** [q)]	**Day 180**	**Day 240**	**Day 360**
Study vaccine administration		▲[g)]				▼[g)]						
Safety Assessments												
Urine pregnancy test [e)]		X[h)]				X[h)]						
Physical examination [j)]	X	X				X			X			
Vital signs [i)]	X	X[i)]				X[i)]						
12-Lead electrocardiogram (ECG) [k)]	X		X				X					
Distribution of subject diary [l)]		X				X						
Review of subject diary by site staff			X	X			X	X				
Adverse events	Continuous											
Serious adverse events	Continuous											
Adverse events related to blood draws	Continuous											
Concomitant medications		X	X	X	X	X	X	X	X	X[m)]		
Clinical Laboratory Samples												
Chemistry, hematology [m)] (mL)	X[o)] (20)	X[h)] (10)	X (10)	X (10)		X[h)] (10)	X (10)	X (10)				
Urinalysis	X[o)]	X[h)]		X		X[h)]		X				
Immunogenicity Blood Samples												
Peripheral blood mononuclear cell (PBMC) assay (mL)		X[h)] (60)		X (40)	X (40)	X[h)] (40)		X (40)	X (100)	X (40)	X (40)	X (40)
Humoral assays (mL)		X[h)] (25)		X (20)	X (20)	X[h)] (20)		X (20)	X (60)	X (20)	X (20)	X (20)
Approximate Blood Volumes												
Daily blood draws (mL)	20	95	10	70	60	70	10	70	160	60	60	60
Cumulative blood draws (mL)	20	115	125	195	255	325	335	405	565	625	685	745

Group 2: ▲MVA-BN-Filo 1x10^8 TCID$_{50}$ or placebo, ▼Ad26.ZEBOV 5x10^{10} vp or placebo *Group 4: ▲ Ad26.ZEBOV 5x10^{10} vp or placebo, ▼ MVA-BN-Filo 1x10^8 TCID$_{50}$ or placebo*

Other footnotes are provided after the Time and Events Schedule for Group 5.

Group 5		Day 1	Day 4	Day 8	Day 15	Day 18	Day 22	Day 36 [q)]	Day 180	Day 240	Day 360
Study vaccine administration		▲ [g)]			▼ [g)]						
Safety Assessments											
Urine pregnancy test [e)]		X [h)]			X [h)]						
Physical examination [j)]	X	X			X			X			
Vital signs [i)]	X	X			X						
12-Lead electrocardiogram [k)]	X		X			X					
Distribution of subject diary [l)]		X			X						
Review of subject diary by site staff			X	X		X	X				
Adverse events	Continuous										
Serious adverse events	Continuous										
Adverse events related to blood draws	Continuous										
Concomitant medications		X	X	X	X	X	X	X	X [m)]		
Clinical Laboratory Assessments											
Hematology, chemistry [n)] (mL)	X [o)] (20)	X [h)] (10)	X (10)	X (10)	X [h)] (10)	X (10)	X (10)				
Urinalysis	X [o)]	X [h)]		X	X [h)]		X				
Immunogenicity Blood Samples											
Peripheral blood mononuclear cell (PBMC) assay (mL)		X [h)] (60)		X (40)	X [h)] (40)		X (40)	X (100)	X (40)	X (40)	X (40)
Humoral assays (mL)		X [h)] (25)		X (20)	X [h)] (20)		X (20)	X (60)	X (20)	X (20)	X (20)
Approximate Blood Volumes											
Daily blood draws (mL)	20	95	10	70	70	10	70	160	60	60	60
Cumulative blood draws (mL)	20	115	125	195	265	275	345	505	565	625	685

Group 5: ▲ *Ad26.ZEBOV* $5x10^{10}$ *vp;* ▼ *MVA-BN-Filo* $1x10^{8}$ $TCID_{50}$. *Other footnotes are provided on the next page.*

NOTE 1: In case of early withdrawal due to an adverse event, the investigator will collect all information relevant to the adverse event and safety of the subject, and will follow the subject until resolution or until reaching a clinically stable endpoint. If feasible, blood will be drawn for immunologic assays. Subjects who wish to withdraw consent will be offered an optional visit for safety follow-up (before the formal withdrawal of consent). The subject has the right to refuse.

NOTE 2: If blood sampling or vital sign measurement is scheduled for the same time point as electrocardiogram (ECG) recording, the procedures should be performed in the following order: ECG, vital signs, blood draw.

a) Screening must be performed within 28 days (Groups 1 to 4) or 56 days (Group 5) before Day 1 and may be split into multiple days or visits. Retesting of values (eg, safety laboratory) that lead to exclusion is allowed once using an unscheduled visit during the screening period, provided there is an alternative explanation for the out of range value.
b) Signing of the informed consent form (ICF) needs to be done before the first study-related activity.
c) The investigators should ensure that all study enrollment criteria have been met at the end of the screening period. If a subject's status changes (including laboratory results or the receipt of additional medical records) after screening but before Day 1 such that the subject no longer meets all eligibility criteria, then the subject should be excluded from participation in the study.
d) Prestudy therapies up to 30 days prior to the start of screening must be recorded in the case report form (CRF).
e) For women of childbearing potential.
f) For women >45 years of age who are postmenopausal for less than 2 years or at any age with amenorrhea for more than 6 months.
g) Subjects will be contacted after at least 24 hours for collection of adverse events.
h) Prior to study vaccine administration.
i) Includes heart rate, blood pressure and oral body temperature. To be assessed immediately prior to study vaccine administration and 30 and 60 minutes after study vaccine administration.
j) A full physical examination, including height and body weight, will be carried out at screening. At other visits, an abbreviated, symptom-directed examination will be performed as indicated by the investigator.
k) A single, 12-lead ECG (supine) after at least 5 minutes rest will be performed at screening and 3 days after each vaccination, and will be read locally. ECGs may be repeated at other time points during the study if clinically indicated based on signs and symptoms.
l) After administration of study vaccine, subjects will be observed directly for 30 minutes for reactogenicity, and remain at the site for a total of 60 minutes post-vaccination to monitor the development of any acute reactions, or longer in case of grade 3 adverse events. Subjects will use the subject diary to document symptoms of unsolicited and solicited local and systemic adverse events in the evening after each vaccination and then daily for the next 7 days.
m) During long-term follow-up, concomitant therapies should be recorded only if given in conjunction with serious adverse events
n) Volumes are the maximum volumes expected to be drawn.
o) Samples at screening may be fasted or non-fasted. If the laboratory sampling occurred more than 28 (main study) or 56 (substudy) days before the baseline visit (Day 1; the day of prime vaccination), sampling will need to be repeated.
p) Only subjects who received active vaccine will enter the long-term follow-up for the collection of serious adverse event information, adverse events related to blood draws, and blood draws for evaluation of the kinetics of immune response over time.
q) In addition to the assessments scheduled for this 21-day post-boost visit, subjects will be instructed to contact the investigator if they experience any adverse event or intercurrent illness that they perceive as relevant and/or can be possibly related to study vaccine in their opinion. In addition, after the main study is unblinded, subjects who received placebo will be contacted to communicate that they have completed the study and do not need to contact the site any longer.

ABBREVIATIONS

Ad	adenoviral vector (serotype indicated by a number, eg, Ad26)
Ad26.ENVA.01	Ad26 vector expressing the human immunodeficiency virus type 1, Clade A envelope protein
Ad26.ZEBOV	Ad26 vector expressing the glycoprotein of the Ebola virus Mayinga variant
ALT	alanine aminotransferase
AST	aspartate aminotransferase
β-hCG	β-human chorionic gonadotropin
BMI	body mass index
BN	Bavarian Nordic
BUN	blood urea nitrogen
CEF	chicken embryo fibroblast
CI	confidence interval
CRF	case report form
CRP	C-reactive protein
DMID	Division of Microbiology and Infectious Diseases
DNA	deoxyribonucleic acid
DRC	Data Review Committee
EBOV	Ebola virus
ECG	electrocardiogram
eDC	electronic data capture
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
ELISpot	enzyme-linked immunospot
Env	envelope
ESR	erythrocyte sedimentation rate
EU	European Union
FA	Full Analysis
FDA	Food and Drug Administration
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GP	glycoprotein
HBsAg	hepatitis B surface antigen
HIV	human immunodeficiency virus
ICF	informed consent form
ICH	International Conference on Harmonisation
ICS	intracellular cytokine staining
IEC	Independent Ethics Committee
IFN	interferon
IgG	immunoglobulin G
IL	interleukin
IM	intramuscular
IR	Immunogenicity Response
IRB	Institutional Review Board
IUD	intrauterine device
IUS	intrauterine hormone-releasing system
IWRS	interactive web response system
kb	kilobase
MARV	Marburg virus
MedDRA	Medical Dictionary for Regulatory Activities
MVA	Modified Vaccinia Ankara
MVA-BN	Modified Vaccinia Ankara - Bavarian Nordic
MVA-BN-Filo	Modified Vaccinia Ankara - Bavarian Nordic vector expressing the glycoproteins of Ebola virus, Sudan virus and Marburg virus and the nucleoprotein of Tai Forest virus
NHP	nonhuman primate
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health

NP	nucleoprotein
PBMC	peripheral blood mononuclear cell
PIR	post-injection reactogenicity
PP	Per Protocol
PQC	Product Quality Complaint
QTcF	Fridericia's corrected QT interval
RNA	ribonucleic acid
SUDV	Sudan virus
SUSAR	serious, unexpected suspected adverse reaction
TAFV	Tai Forest virus
$TCID_{50}$	50% Tissue Culture Infective Dose
THAM	tris (hydroxymethyl)-amino methane
TNF	tumor necrosis factor
TOPS	The Over-volunteering Prevention System
US	United States
VISP	vaccine induced seropositivity
vp	viral particles
WHO	World Health Organization

DEFINITIONS OF TERMS

Active vaccine	MVA-BN-Filo or Ad26.ZEBOV
Study vaccine	MVA-BN-Filo, Ad26.ZEBOV or placebo
Independent study vaccine administrator	A trained study nurse, medical doctor, or otherwise qualified healthcare provider who will have no other study function
Solicited adverse events (reactogenicity)	Local and systemic adverse events that are common and known to occur after vaccination and that are usually collected in a standard, systematic format in vaccine clinical studies. For the list of solicited adverse events in this study, see Section 9.3.2. For the purpose of vaccine clinical studies, all other adverse events are considered unsolicited; however, this definition should be distinguished from definitions based on pharmacovigilance guidelines.

1. INTRODUCTION

Crucell Holland BV is investigating the potential of a prophylactic Ebola vaccine regimen comprising the following 2 candidate Ebola vaccines:

Ad26.ZEBOV is a monovalent vaccine expressing the full length Ebola virus (EBOV, formerly known as *Zaire ebolavirus*) Mayinga glycoprotein (GP), and is produced in the human cell line PER.C6®.

MVA-mBN226B (further referred to as MVA-BN-Filo®) is a multivalent vaccine expressing the Sudan virus (SUDV) GP, the EBOV GP, the Marburg virus (MARV) Musoke GP, and the Tai Forest virus (TAFV, formerly known as *Côte d'Ivoire ebolavirus*) nucleoprotein (NP). The EBOV GP expressed by MVA-BN-Filo has 100% homology to the one expressed by Ad26.ZEBOV.

For the most up-to-date nonclinical and clinical information regarding Ad26.ZEBOV and MVA-BN-Filo, refer to the latest versions of the Investigator's Brochures and Addenda (if applicable).[12,13,14]

The term "sponsor" used throughout this document refers to the entities listed in the Contact Information page(s), which will be provided as a separate document.

1.1. Background

Ebola viruses belong to the Filoviridae family and cause Ebola disease, which can induce severe hemorrhagic fever in humans and nonhuman primates (NHPs). Case fatality rates in Ebola disease range from 25% to 90% (average: 50%), according to the World Health Organization (WHO).[25] These viruses are highly prioritized by the United States (US) Government, who has defined them as 'Category A' agents, due to the high mortality rate of infected individuals. Currently, no licensed vaccine, treatment or cure exists for this disease.

Filoviruses are named for their long, filamentous shape. Within this filamentous virus, a single 19-kilobase (kb) negative-sense ribonucleic acid (RNA) genome encodes 7 proteins: the GP, the polymerase, the NP, the secondary matrix protein, the transcriptional activator, the polymerase cofactor, and the matrix protein. The virion surface is covered by homotrimers of the viral GP, which is believed to be the sole host attachment factor for filoviruses. Following cell entry, the viruses replicate their genomes and viral proteins in the cytoplasm using a RNA-dependent RNA polymerase, which is carried into the cell together with the virus.[9]

Crucell Holland BV, The Netherlands, in collaboration with Bavarian Nordic, Denmark, and in partnership with the Division of Microbiology and Infectious Diseases (DMID) of the National Institute of Allergy and Infectious Diseases (NIAID) - National Institutes of Health (NIH), is developing a vaccine regimen targeting the EBOV species, which is responsible for the Ebola disease outbreak that started in December 2013 in Guinea and further disseminated in West Africa. The monovalent vaccine is part of an ongoing development program for a multivalent vaccine against multiple filoviruses that cause disease in humans, including EBOV, SUDV and MARV.

This first-in-human study consists of 2 parts. The first part, ie, the main study, is a randomized, placebo-controlled, observer-blind study, and will evaluate the Crucell adenovirus serotype 26 (Ad26) vector expressing the GP of the EBOV Mayinga variant (Ad26.ZEBOV) and the Modified Vaccinia Ankara (MVA) - Bavarian Nordic (BN) vector with EBOV, SUDV and MARV GP inserts and TAFV NP insert (MVA-BN-Filo)as a heterologous prime-boost regimen, in which each vaccine is used to prime a filovirus-specific immune response followed by a boost immunization with the other vaccine 28 or 56 days later. The second part is an open-label, uncontrolled substudy that will evaluate a regimen in which Ad26.ZEBOV is used as a prime, and MVA-BN-Filo is given as a boost 14 days later. The GP of the EBOV virus that is currently circulating in West Africa has 97% homology to the EBOV GP used in this vaccine regimen.[4]

Nonclinical Studies

Immunogenicity and Efficacy

Immunogenicity and efficacy of the vaccine combination Ad26.ZEBOV and MVA-BN-Filo was evaluated in an NHP model (ie, Cynomolgus macaques, *Macaca fascicularis*). The combination was assessed in a multivalent filovirus setting in a small number (2 per regimen) of animals and the study included heterologous prime-boost regimens of Ad26, serotype 35 (Ad35) and MVA-BN-Filo vectors expressing different Ebola and Marburg proteins. Full protection from Ebola disease and death after wild-type EBOV Kikwit 1995 challenge was obtained with all heterologous regimens, including the Ad26 and MVA vaccine regimen. All heterologous prime-boost regimens induced comparable immune responses against the EBOV Mayinga GP. Independently of the vaccine regimen, a strong boost effect was seen after heterologous prime-boost immunization. An additional study involving more animals is planned, to strengthen the robustness of the nonclinical efficacy data, and also to optimize the prime-boost schedule so as to obtain induction of protective immunity as quickly as possible, to specifically respond to the Ebola disease outbreak that is currently occurring in West Africa.

Toxicology

A repeated-dose toxicity study in rabbits is being conducted to support the present first-in-human study with prime-boost combinations of Ad26.ZEBOV and MVA-BN-Filo. The objective of this study is to assess potential toxicity and local tolerance of the vaccines when given to rabbits by intramuscular (IM) injection on Days 1 and 15/16, and to evaluate the reversibility, persistence or delayed occurrence of any adverse effects of the vaccines over a 14-day recovery period after the last injection. Part of the data from this study (all data for 25% of animals and all data minus histopathology for another 25% of animals) will be available prior to the start of enrollment for the present study.

Supportive toxicity studies in rabbits performed to date using the Ad26 vector in combination with other inserts or Ebola GP inserts in combination with other vectors have shown good tolerability and no toxicologically relevant findings.

A preliminary report of safety assessments in NHPs vaccinated with heterologous prime-boost regimens involving Ad26, Ad35 and MVA-BN-Filo vectors expressing different Ebola and

Marburg proteins, indicates that the NHPs appeared to tolerate the vaccination, without signs of adverse effects.

Biodistribution

Single-dose biodistribution studies in rabbits were performed using the MVA-BN vector or the Ad26 vector in combination with another insert (Ad26.ENVA.01: an experimental, prophylactic Ad26 vector expressing the human immunodeficiency virus [HIV] type 1, Clade A envelope protein). Neither vector distributed widely upon IM inoculation. MVA-BN distributed to the skin, muscle, blood, spleen, lung, liver, and pooled lymph nodes and was rapidly cleared (within 48 hours following vaccination). Ad26.ENVA.01 was primarily localized in the injection site muscle, the regional lymph nodes and the spleen. Three months after the single IM injection of Ad26.ENVA.01, the vaccine was cleared from most of the examined tissues. As biodistribution is dependent on the vector platform (MVA or Ad26) and not on the insert, it can be assumed that recombinant MVA-BN-Filo or Ad26.ZEBOV is distributed in the same way as the MVA-BN vector or Ad26.ENVA.01 vector, respectively.

Clinical Studies

There is no previous clinical experience with either MVA-BN-Filo or Ad26.ZEBOV. Safety data generated with the 2 vaccines with different inserts are provided below.

Safety Profile

Ad26.ZEBOV is a monovalent recombinant, replication-incompetent Ad26-based vector. Clinical data for Ad26.ZEBOV are not yet available. However, adenovirus vaccine programs with other gene inserts have revealed no significant safety issues. The data described below are based on the evaluation of the prototype vaccine Ad26.ENVA.01, which expresses the HIV Envelope (Env) gene.[15]

Three randomized, placebo-controlled, Phase 1 studies (IPCAVD-001, IPCAVD-003, IPCAVD-004) have evaluated the safety and immunogenicity of the prototype vaccine Ad26.ENVA.01. This prototype vaccine has been administered to more than 200 healthy, HIV-negative, healthy subjects between the ages of 18 and 50 years in the US and Africa:[15,16,17]

- In the dose-escalation study IPCAVD-001 (n=60), 2 or 3 IM doses of Ad26.ENVA.01 ($1x10^9$, $1x10^{10}$, $5x10^{10}$, $1x10^{11}$ viral particles [vp]) were given to Ad26 seronegative subjects. There were no deaths or vaccine-related serious adverse events. Ad26.ENVA.01 was generally well tolerated at all 4 dose levels with minimal reactogenicity observed in the $1x10^9$ and $1x10^{10}$ vp dose groups. Moderate to severe malaise, myalgia, fatigue and chills occurred in the majority of subjects 12 to 18 hours after the first dose of $1x10^{11}$ vp, but were resolved within 24 to 36 hours and were not seen after the second injection at this dose level. Two subjects in the $1x10^{11}$ vp dose group chose not to have the second injection, however, one of them decided to have the 6-month injection. Envelope-specific humoral and cell-mediated immune responses were induced at all 4 dose levels of vaccine.[3,5]
- In the single-dose study IPCAVD-003 (n=24), an IM dose of Ad26.ENVA.01 ($5x10^{10}$ vp) or placebo was given to subjects, who were stratified according to baseline Ad26 immune status, to evaluate the safety, mucosal immunogenicity and innate immune responses. Local

reactogenicity comprised moderate injection site pain/tenderness and/or moderate to severe erythema which resolved within 3 days of vaccination. Transient systemic reactogenicity comprised headache, chills, joint pain, myalgia, malaise/fatigue, and fever. No deaths or vaccine-related serious adverse events were observed. Vaccination elicited both systemic and mucosal Env-specific humoral and cellular immune responses. No increased activated total or vector-specific mucosal CD4 T-lymphocytes following vaccination were detected in the colorectal mucosa, indicating that vaccination with Ad26 did not increase mucosal inflammation.[1,2]

- In study IPCAVD-004 (n=217), the safety and immunogenicity of IM doses of Ad26.ENVA.01 and Ad35.ENV (an Ad35 vector expressing an HIV envelope GP used in that study at a dose of $5x10^{10}$ vp), given in heterologous and homologous prime-boost regimens at 3- versus 6-month intervals, was evaluated. There were 452 adverse events reported by 84 of 176 Ad26-vaccine recipients (47.7%), the majority being mild (75.5%) in severity. The proportion of subjects with moderate or severe symptoms was not statistically significantly different between vaccine and placebo. There were 3 serious adverse events: 2 serious adverse events in placebo recipients (grade 3 peritonsillar abscess and grade 4 migraine headache, both resolved with no residual effects) and 1 serious adverse event in an Ad35/Ad26 vaccine recipient (grade 4 acute myelogenous leukemia, resolved with sequelae). No deaths or vaccine-related serious adverse events were reported. Overall, 97% to 100% of subjects developed anti-Env binding antibodies (enzyme-linked immunosorbent assay [ELISA]) after a second dose, with heterologous and homologous regimens being comparable. Immune responses in groups who received 3-month and 6-month schedules were comparable. Four weeks post-vaccination, interferon (IFN)-γ enzyme-linked immunospot (ELISpot) showed response rates between 44% and 100%. The heterologous and homologous regimens were comparable. There was induction of Ad26-neutralizing antibodies in the majority of vaccine recipients after 2 immunizations with Ad26.ENVA.01.[19]

In addition, a Phase 1/2a double-blind, randomized, placebo-controlled, dose-escalation study sponsored by Crucell (MAL-V-A001) evaluated the safety, tolerability and immunogenicity of 2 dose levels ($1x10^{10}$ and $5x10^{10}$ vp) of Ad35.CS.01/Ad26.CS.01 (both expressing the malaria *Plasmodium falciparum* circumsporozoite antigen) prime-boost regimens in healthy subjects. The dose escalation phase was followed by an evaluation of efficacy of the higher dose level in an experimental malaria challenge. A total of 42 subjects were enrolled and were vaccinated. The analysis of adverse events did not show any consistent pattern suggestive of an association of Ad35.CS.01 or Ad26.CS.01 with specific adverse events. There were no serious adverse events reported during the study. No subject discontinued during a study phase (vaccination or challenge) due to adverse events. One subject in the high-dose group completed the vaccination phase and the final safety follow-up visit but did not take part in any challenge phase activities because of ongoing dyspnea. The most common related adverse events after each vaccination were injection site pain, malaise, headache, myalgia and chills. The incidence of vaccine-related adverse events was generally higher in the high-dose group than in the low-dose group. In general, incidence of malaise, headache, and myalgia were higher after the third dose (Ad26) than after the first or second doses (Ad35). Injection site pain was more commonly reported in the low and high-dose groups than by placebo subjects. There were no clinically significant changes in laboratory test parameters or vital signs data.[6]

Recent data indicate that administration of a deoxyribonucleic acid (DNA) vaccine expressing EBOV Mayinga GP, the same GP as in the Ad26.ZEBOV component, was safe, well tolerated and immunogenic in a Phase 1 clinical study. During this study, 9 subjects received three 4-weekly IM doses of vaccine (4 mg/dose), followed up by a boost at ≥32 weeks in 8 subjects.[21]

Based on the previous clinical experience of Ad26 vector with different inserts, there has been no impact of Ad26 seropositivity on subjects' safety and only limited impact on immunogenicity results. Therefore, there are no safety concerns with regard to the inclusion of Ad26 seropositive subjects in the study, and the study subjects will not be screened for Ad26 seropositivity as part of the study eligibility criteria. The purpose of the Ad26 seropositivity assessment at baseline is to evaluate its impact, if any, on vaccine immunogenicity.

Safety Profile of MVA-BN

MVA-BN is a further attenuated version of the MVA virus, which in itself is a highly attenuated strain of the poxvirus Chorioallantois Vaccinia Virus Ankara. MVA-BN induces strong cellular activity as well as a humoral (antibody) immune response and has demonstrated an ability to stimulate a response even in individuals with pre-existing immunity against Vaccinia. One of the advantages of MVA-BN is the virus' inability to replicate in a vaccinated individual. The replication cycle is blocked at a very late stage, which ensures that new viruses are not generated and released. This means that the virus cannot spread in the vaccinated person and none of the serious side effects normally associated with replicating Vaccinia viruses have been seen with MVA-BN.

MVA-BN (MVA-BN®, trade name IMVAMUNE® outside the European Union [EU], invented name IMVANEX® in the EU) has received marketing authorization in Canada and the EU for active immunization against smallpox in adults, including immunocompromised subjects in whom traditional replicating vaccines are contraindicated.[11] A Phase 3 clinical study is ongoing in the US (ClinicalTrials.gov Identifier: NCT01144637). Results of completed and ongoing clinical studies of MVA-BN-based vaccines in more than 7,500 individuals, including elderly, children and immunocompromised subjects in whom replicating vaccines are contraindicated, have shown that the platform displays high immunogenicity and a favorable safety profile.[14] Across all clinical studies, no trends for unexpected or serious adverse reactions due to the product were detected. MVA-BN and MVA-BN-based recombinant vaccines have shown to be safe in healthy subjects as well as in immunocompromised subjects in whom traditional replicating vaccines are contraindicated, eg, individuals with HIV infection or diagnosed with atopic dermatitis.

Extensive nonclinical studies support the safety profile of the MVA-BN strain.[22,23]

Viral Shedding

Viral shedding information is available from 6 clinical studies with adenovirus vectored vaccines against HIV type 1 (using Ad26 and Ad35: Ad26.ENVA.01 and Ad35.ENV) and *Mycobacterium tuberculosis* (using Ad35: AERAS-402). Viral shedding was not observed in any of these clinical studies. In a clinical study evaluating viral shedding of Ad26.ENVA.01 and Ad35.ENV (Study IPCAVD-004), all cultures from oropharyngeal swabs and urine were negative for adenovirus; in

5 clinical studies evaluating viral shedding of AERAS-402 (Studies C-001-402, C-003-402, C-008-402, C-009-402, C-017-402), no shedding of AERAS-402 was seen in any of the urine or throat cultures.[12]

MVA-BN-Filo is an attenuated recombinant MVA incapable of replication in human cells with a block in the late stage of virus replication. In human cells, upon infection, viral genes are expressed, but no infectious progeny virus is produced. Given the inability of virus assembly and very limited host range of the vector, no viral shedding studies were performed.

1.2. Risk Benefit Section

1.2.1. Known Benefits

The clinical benefit of prime-boost combinations of Ad26.ZEBOV and MVA-BN-Filo is to be established.

1.2.2. Potential Benefits

Subjects may benefit from clinical testing and physical examination; others may benefit from knowledge gained in this study that may aid in the development of an Ebola vaccine.

1.2.3. Known Risks

See the safety data presented in Section 1.1, Background.

1.2.4. Potential Risks

The following potential risks for Ad26.ZEBOV and MVA-BN-Filo will be monitored during the study and are specified in the protocol:

Risks Related to Vaccines

Subjects may exhibit general signs and symptoms associated with administration of a vaccine, or a placebo vaccination, including fatigue, nausea, headache, vomiting, myalgia, rash, arthralgia, general itching, fever, and chills. In addition, subjects may experience local (injection site) reactions such as pain/tenderness, erythema, induration, swelling, itching and/or warmth at the injection site. These events will be monitored, but are generally short-term and do not require treatment.

Subjects may have an allergic reaction to the vaccination. An allergic reaction may cause a rash, hives or even difficulty breathing. Severe reactions are rare. Medications must be available in the clinic to treat serious allergic reactions.

The risks related to vaccine induced seropositivity (VISP) are discussed in Section 9.4, Vaccine Induced Seropositivity.

Risk of Myo/Pericarditis

While replicating smallpox vaccines have been associated with an increased risk to develop myo/pericarditis,[20] this has not been observed with MVA-BN and is not expected with this highly

attenuated, non-replicating vaccine. Based on observations with first- and second-generation replication-competent smallpox vaccines, particular attention has been placed on the monitoring for cardiac signs and symptoms in all clinical studies using MVA-BN. Despite the close cardiac monitoring, no event indicating a case of myo/pericarditis has been observed in any completed MVA-BN study. In a review of prospective surveillances for cardiac adverse events in 6 different clinical studies in 382 subjects receiving MVA vaccines, only 1 subject (0.3%) met the criteria for vaccine-induced myocarditis and eventually the subject was found to suffer from exercise-induced palpitations. Self-limited mild elevations in troponin I were recorded in 3 (0.8%) subjects without evidence of myo/pericarditis.[7]

For the present study, subjects will be actively screened to exclude pre-existing cardiac concerns and enrolled subjects will be monitored during the study.

Pregnancy and Birth Control

The effect of the study vaccines on a fetus or nursing baby is unknown, as well as the effect on semen, so women of childbearing potential, and men having sexual intercourse with women, are required to agree to practice adequate birth control measures for sexual intercourse from 28 days before the prime vaccination until at least 3 months after the boost vaccination (see Section 9.1.3, Screening). Women who are pregnant or breast-feeding, or are planning to become pregnant while enrolled in the study or within 3 months after the boost vaccination, will be excluded from enrollment into the study.

Women of childbearing potential must also agree not to donate eggs (ova, oocytes) for the purposes of assisted reproduction from the start of screening onwards until at least 3 months after the boost vaccination. Men must also agree not to donate sperm from the start of screening onwards until at least 3 months after the boost vaccination.

Note: after the main study is unblinded, placebo subjects may stop birth control measures or resume their pre-study birth control measures, and may donate eggs (females) or sperm (males).

Risks from Blood Draws

Blood draws may cause pain, bruising, bleeding, and, rarely, infection at the site where the blood is taken.

Unknown Risks

There may be other serious risks that are not known.

1.2.5. Overall Benefit/Risk Assessment

Based on the available data and proposed safety measures, the overall risk/benefit assessment for this clinical study is acceptable for the following reasons:

- There is no previous clinical experience with MVA-BN-Filo or Ad26.ZEBOV. However, safety data generated with the 2 vaccines with different inserts have revealed no significant safety issues (see Section 1.1).

- Only subjects who meet all inclusion criteria and none of the exclusion criteria (specified in Sections 4.1 and 4.2, respectively) will be allowed to participate in this study. The selection criteria include adequate provisions to minimize the risk and protect the well-being of subjects in the study.
- Safety will be closely monitored throughout the study:
  - After each vaccination, subjects will be observed directly for 30 minutes and remain at the site for a total of 60 minutes post-vaccination to monitor the development of any acute reactions, or longer in case of grade 3 adverse events. Subjects will use a diary to document symptoms of unsolicited and solicited local and systemic adverse events in the evening after each vaccination and then daily for the next 7 days.
  - The investigator or the designee will document unsolicited adverse events from signing of the informed consent form (ICF) onwards until 21 days post-boost, and serious adverse events and adverse events related to blood draws from signing of the ICF onwards until the end of the study.
  - Safety measures, including laboratory tests, vital sign measurements, electrocardiograms (ECGs), and physical examinations, will be performed at scheduled visits during the study.
  - Any clinically significant abnormalities persisting at the end of the study or upon early withdrawal will be followed by the investigator until resolution or until a clinically stable endpoint is reached.
- Several safety measures are included in this protocol to minimize the potential risk to subjects, including the following:
  - The safety evaluations described in Section 0 take into account adverse events of interest based on clinical safety data and available nonclinical data.
  - There are predefined pausing rules (see Section 11.8) that would result in pausing of further vaccination if predefined conditions occur, preventing exposure of new subjects to study vaccine until an internal Data Review Committee (DRC) reviews all safety data. Study enrollment would be resumed only if the DRC recommends it.
  - Subjects will be withdrawn from study vaccine administration for the following reasons (see also Section 10.2, Discontinuation of Study Vaccine):
    - Any reason listed in Section 11.8, Pausing Rules, preventing a subject from receiving boost vaccination
    - Pregnancy
    - Any adverse event considered at least possibly related to the study vaccine, worsening of health status or intercurrent illnesses that, in the opinion of the investigator, requires discontinuation from study vaccine
    - Confirmed Ebola disease
    - Use of disallowed medications listed in Section 8, Prestudy and Concomitant Therapy.

- If acute illness (excluding minor illnesses such as diarrhea or mild upper respiratory tract infection) or fever (oral temperature ≥38.0 °C) occur at the scheduled time for vaccination, the subject may be vaccinated up to 10 days beyond the window allowed for the scheduled vaccination, or be withdrawn from that vaccination at the discretion of the investigator and after consultation with the sponsor (see Section 10.3).
- If a subject withdraws from the study (withdrawal of consent), he/she maintains the option to participate in the safety follow-up.

1.3. Overall Rationale for the Study

In nonclinical studies in the Cynomolgus macaque model, heterologous prime-boost regimens of a multivalent mixture of Ad26 vectors (each expressing EBOV Mayinga, SUDV or MARV GP) and MVA-BN-Filo provided complete protection against the highly pathogenic wild-type EBOV Kikwit 1995 variant (report pending). Further nonclinical studies are ongoing to evaluate the protection of the multivalent vaccine regimen in additional animals and to assess the protective efficacy of a combination regimen of Ad26.ZEBOV and MVA-BN-Filo (either a simultaneous administration or as prime-boost regimen).

In humans, both Ad26- and MVA-based vaccines containing various antigenic inserts have been shown to be safe and immunogenic (see Section 1.1, Background). To date, more than 200 subjects have received Crucell Ad26-based vaccines in clinical studies. The MVA-BN platform is the basis of the non-replicating smallpox vaccine registered in Canada and Europe, and has been safely used in more than 7,500 humans.[14] Although routinely used by the subcutaneous route, MVA-BN at a dose of $1x10^8$ $TCID_{50}$ (50% Tissue Culture Infective Dose) has been demonstrated to be as safe and immunogenic when used by the IM route.[8,24] The IM route has been chosen for the present study for blinding purposes.

The unprecedented size and scale of the ongoing Ebola disease outbreak that started in December 2013 in Guinea and subsequently spread to Sierra Leone, Nigeria and Liberia, led to the outbreak being declared a public health emergency of international concern in August 2014 by the WHO. This study is one of a series of studies to evaluate the heterologous combination of Ad26.ZEBOV and MVA-BN-Filo as a possible vaccine regimen to prevent Ebola disease. It will test schedules that will be evaluated in planned NHP challenge studies.

2. OBJECTIVES AND HYPOTHESIS

2.1. Objectives

This first-in-human study aims to evaluate the safety, tolerability and immunogenicity of different MVA-BN-Filo and Ad26.ZEBOV vaccine regimens.

Primary Objectives

- To assess the safety and tolerability of heterologous prime-boost regimens utilizing MVA-BN-Filo at a dose of $1x10^8$ $TCID_{50}$ and Ad26.ZEBOV at a dose of $5x10^{10}$ vp.

Secondary Objectives

- To assess humoral and cellular immune responses to the EBOV GP of the various regimens tested as measured by virus neutralization assay, ELISA and ELISpot assay.

Exploratory Objectives

- To explore further humoral and cellular immune responses to the EBOV GP and the adenovirus and MVA backbone of the various regimens tested. The assays to be used may include, but will not be limited to, adenovirus/MVA neutralization assays, molecular antibody characterization, and intracellular cytokine staining (ICS).
- To explore humoral and cellular immune responses to the SUDV GP, MARV GP and TAFV NP if assays are available.

2.2. Hypothesis

No formal statistical hypothesis testing is planned.

3. STUDY DESIGN AND RATIONALE

3.1. Overview of Study Design

This Phase 1 first-in-human study consists of 2 parts.

The main study is a randomized, placebo-controlled, observer-blind study evaluating the safety, tolerability and immunogenicity of 4 regimens using MVA-BN-Filo at a dose of $1x10^8$ $TCID_{50}$ and Ad26.ZEBOV at a dose of $5x10^{10}$ vp: 2 regimens will have MVA-BN-Filo as prime and Ad26.ZEBOV as boost at a 28- or 56-day interval, and 2 regimens will have Ad26.ZEBOV as prime and MVA-BN-Filo as boost at a 28- or 56-day interval. The main study will be conducted in approximately 72 healthy adult subjects who never received an experimental Ebola candidate vaccine before and have no known exposure to or diagnosis of Ebola disease.

The substudy consists of an open-label, uncontrolled non-randomized treatment arm evaluating the safety, tolerability and immunogenicity of a regimen with Ad26.ZEBOV at a dose of $5x10^{10}$ vp as prime, and MVA-BN-Filo at a dose of $1x10^8$ $TCID_{50}$ as boost 14 days later, and will be conducted in approximately 15 healthy adult subjects.

The study consists of a screening period (of up to 28 days in the main study and up to 56 days in the substudy), a vaccination period in which subjects will be vaccinated at baseline (Day 1) followed by a boost on Day 15, 29 or 57, and a post-boost follow-up until all subjects have had their 21-day post-boost visit (Day 36, 50 or 78) or discontinued earlier. The main study will be unblinded when all subjects in the main study have had their 21-day post-boost visit or discontinued earlier. After unblinding, subjects who received placebo will be contacted to communicate that they have completed the study and do not need to contact the site any longer. The subjects who received active vaccine will enter a long-term follow-up, with visits on Day 180 (±15 days), Day 240 (±30 days) and Day 360 (±30 days) post-prime.

The Principal Investigator (who is blinded to treatment in the main study) will be responsible for the safety monitoring of the study. If at least one pre-specified pausing rule is met, vaccinations will be paused and an internal DRC meeting will be convened. For details on the DRC, see Section 11.7. For details on the pausing rules, see Section 11.8.

Subjects in the main study will be enrolled into 4 different groups of 18 healthy subjects each. Overall, subjects will be randomized within group in a 5:1 ratio to receive active vaccine or placebo (0.9% saline) through IM injections (0.5 mL) as follows:

- MVA-BN-Filo ($1x10^8$ $TCID_{50}$) on Day 1, followed by a booster of Ad26.ZEBOV ($5x10^{10}$ vp) on Day 29 (Group 1) or Day 57 (Group 2), or
- Ad26.ZEBOV ($5x10^{10}$ vp) on Day 1, followed by a booster of MVA-BN-Filo ($1x10^8$ $TCID_{50}$) on Day 29 (Group 3) or Day 57 (Group 4).

Enrollment of subjects in Groups 1 and 3 will start with vaccination of 1/1 subjects (active vaccine/placebo; Sentinel Cohort) to assess the tolerability of the 2 study vaccines over a 24-hour period before exposing larger cohorts of subjects to the vaccines. Subjects will be contacted by telephone after at least 24 hours to verify the absence of any predefined events (ie, a serious adverse event considered to be related to any of the study vaccines, signs of anaphylaxis or generalized urticaria clearly attributable to study vaccination, a severe [grade 3] unsolicited adverse event considered to be related to any of the study vaccines, a severe [grade 3] solicited injection site reaction, a severe [grade 3] solicited systemic adverse event considered to be related to any of the study vaccines, or death). Enrollment of the next cohorts will be as follows:

- Groups 1 and 3: 4/1 subjects (active vaccine/placebo; Cohort 1) followed, in the absence of any of the above-mentioned events, confirmed by a telephone call with each subject after at least 24 hours after the prime vaccination, by 10/1 subjects (active vaccine/placebo; Cohort 2)
- Groups 2 and 4: 5/1 subjects (active vaccine/placebo; Cohort 1) followed, in the absence of any of the above-mentioned events, confirmed by a telephone call with each subject after at least 24 hours after the prime vaccination, by 10/2 subjects (active vaccine/placebo; Cohort 2)

The enrollment of all subjects of Cohort 1 will be carried out ideally on the same day. Also the enrollment of all subjects of Cohort 2 will be carried out ideally on the same day. If enrollment of Cohort 1 is carried out over more than 1 day, enrollment of Cohort 2 in that group will only start after at least 24 hours after the prime vaccination in the last subject in Cohort 1.

Enrollment in the open-label substudy (Cohort 3) will start after the prime vaccination in the last subject in Cohort 2. Subjects will receive active vaccine through IM injections (0.5 mL) as follows:

- Ad26.ZEBOV ($5x10^{10}$ vp) on Day 1, followed by a booster of MVA-BN-Filo ($1x10^8$ $TCID_{50}$) on Day 15 (Group 5).

Groups 2 and 4 will test a schedule that has already provided protection in an NHP challenge study (though with a higher dose of MVA-BN-Filo [ie, $5x10^8$ $TCID_{50}$] and with Ad26-Tri, which is a trivalent candidate vaccine expressing the EBOV Mayinga, SUDV and MARV GPs) and will therefore serve as reference schedule. Groups 1 and 3 will test a shorter schedule that will be tested in planned NHP challenge studies. Group 5 will explore a schedule that may be further evaluated in future studies.

The study enrollment plan is summarized in Table 6. The different study vaccination schedules are summarized in Table 7.

Table 6: Study Enrollment Plan

Group	Sentinel Cohort	Cohort 1 [a)]	Cohort 2 [b)]	Cohort 3 [c)]
1	1:1 (active vaccine/placebo)	4:1 (active vaccine/placebo)	10:1 (active vaccine/placebo)	
2	-	5:1 (active vaccine/placebo)	10:2 (active vaccine/placebo)	
3	1:1 (active vaccine/placebo)	4:1 (active vaccine/placebo)	10:1 (active vaccine/placebo)	
4	-	5:1 (active vaccine/placebo)	10:2 (active vaccine/placebo)	
5	-	-	-	15 (active vaccine)

a) Cohort 1 will be enrolled after at least 24 hours after the prime vaccination in the Sentinel Cohorts.
b) Cohort 2 will be enrolled after at least 24 hours after the prime vaccination in the last subject in Cohort 1.
c) Cohort 3 will be enrolled after the prime vaccination in the last subject in Cohort 2 for operational/logistic reasons.

Table 7: Study Vaccination Schedules

Group	N		Day 1	Day 15	Day 29	Day 57
1	18	15	MVA-BN-Filo $1x10^8$ $TCID_{50}$	-	Ad26.ZEBOV $5x10^{10}$ vp	-
		3	placebo (0.9% saline)	-	placebo (0.9% saline)	-
2	18	15	MVA-BN-Filo $1x10^8$ $TCID_{50}$	-	-	Ad26.ZEBOV $5x10^{10}$ vp
		3	placebo (0.9% saline)	-	-	placebo (0.9% saline)
3	18	15	Ad26.ZEBOV $5x10^{10}$ vp	-	MVA-BN-Filo $1x10^8$ $TCID_{50}$	-
		3	placebo (0.9% saline)	-	placebo (0.9% saline)	-
4	18	15	Ad26.ZEBOV $5x10^{10}$ vp	-	-	MVA-BN-Filo $1x10^8$ $TCID_{50}$
		3	placebo (0.9% saline)	-	-	placebo (0.9% saline)
5	15		Ad26.ZEBOV $5x10^{10}$ vp	MVA-BN-Filo $1x10^8$ $TCID_{50}$	-	-

N: number of subjects to receive study vaccine; $TCID_{50}$: 50% Tissue Culture Infective Dose; vp: viral particles

After each vaccination, subjects will be observed directly for 30 minutes and remain at the site for a total of 60 minutes post-vaccination to monitor for the development of any acute reactions, or longer in case of grade 3 adverse events. Subjects will be instructed to contact the investigator

immediately if they experience any adverse event that they perceive as relevant or possibly related to study vaccine in their opinion, and will be contacted after at least 24 hours for collection of adverse events and to confirm that no pre-specified pausing rules have been met. Subjects will use a diary to document symptoms of unsolicited and solicited local and systemic adverse events in the evening after each vaccination and then daily for the next 7 days. The investigator or the designee will document unsolicited adverse events from signing of the ICF onwards until 21 days post-boost, and serious adverse events and adverse events related to blood draws from signing of the ICF onwards until the end of the study. In addition, the investigator or designee will collect samples for safety assessments (hematology, chemistry, and urinalysis) and assessments of immune responses, at the time points indicated in the Time and Events Schedule.

The final analysis will be performed when all subjects have completed the last study-related visit or discontinued earlier.

Subjects will be approached to consent for enrollment into the VAC52150 Vaccine Development Registry Protocol for long-term surveillance after completing the present study (see Section 9.1.7 for details).

3.2. Study Design Rationale

Dose Selection

In NHP challenge studies, a prime immunization with a trivalent mix of Ad26 vectors expressing the SUDV GP, the EBOV GP and the MARV GP each at a dose of $4x10^{10}$ vp combined with MVA-BN-Filo at a dose of $5x10^{8}$ $TCID_{50}$ provided full protection in NHP challenge. Importantly, current data suggest that there is no cross-protection between Ebola viruses (Ebola and Sudan) or between Ebola viruses and Marburg viruses.[10,18] Therefore, the protection afforded with a trivalent vaccine can likely be extended to an Ebola monovalent vaccine.

In clinical studies where the Ad26 vector was used with different constructs at doses from $1x10^{9}$ to $1x10^{11}$ vp a dose-dependent increase in immunogenicity was apparent up to a dose of $5x10^{10}$ vp, with only marginal increase in immunogenicity at a dose of 10^{11} vp. Ad26 constructs were generally well tolerated at doses up to $5x10^{10}$ vp while moderate to severe systemic adverse events occurred in some subjects after the first dose of $1x10^{11}$ vp, though resolving within 24 to 36 hours.

A dose of $5x10^{10}$ vp for Ad26.ZEBOV has been selected as the one offering the best risk/benefit ratio in terms of expected immunogenicity and good tolerability.

The MVA-BN smallpox vaccine has been used in clinical and nonclinical development at a dose with a nominal titer of $1x10^{8}$ $TCID_{50}$ and is licensed with an at least titer of $5x10^{7}$ $TCID_{50}$. The dose of $1x10^{8}$ $TCID_{50}$ has been used for other MVA-BN based vectors expressing different inserts which has been demonstrated also safe and immunogenic, while a slight increase in reactogenicity was observed at the higher dose of $5x10^{8}$ $TCID_{50}$, without compromising the overall safety profile. The dose of $1x10^{8}$ $TCID_{50}$ is considered to be the most favorable regarding vaccine availability, immunogenicity and safety profile.

Control and Blinding

Main Study

A placebo control will be used to compare the safety and tolerability and establish the frequency and magnitude of changes in clinical and immunologic endpoints that may occur in the absence of the active vaccine. Observer-blind treatment will be used to reduce potential bias during data collection and evaluation of clinical safety endpoints. Blinding will be guaranteed by preparation of study vaccine by an independent unblinded pharmacist or qualified staff member and by the administration of vaccine in a masked syringe by an independent study vaccine administrator (see Definitions of Terms).

The rationale for defining the main study as an observer-blind study is because the subjects and staff will not be blinded to the schedule, ie, no additional placebo injections will be administered to mask the regimens across groups. Blinding will therefore only be within groups.

In view of the ongoing Ebola disease outbreak, some cohorts will be enrolled in parallel.

Substudy

All subjects in Group 5 will receive the same active vaccine regimen. The sample size of 15 subjects in Group 5 aligns with 15 subjects on active vaccine in each group in the main study (Groups 1 to 4). The substudy is open-label since the placebo-controlled main study with a total of 60 subjects on active treatment is considered sufficient to provide a preliminary assessment of safety and immunogenicity. Furthermore, results of Group 5 will be analyzed separately from results of Groups 1 to 4. Blinding and randomization procedures are not applicable for the subjects included in Group 5.

4. SUBJECT POPULATION

Screening for eligible subjects in the main study must be performed within 28 days before Day 1, and within 56 days before Day 1 in the substudy.

The inclusion and exclusion criteria for enrolling subjects into this study are described in the following 2 subsections. If there is a question about the inclusion or exclusion criteria below, the investigator should consult with the appropriate sponsor representative before enrolling a subject in the study.

For a discussion of statistical considerations of subject selection, see Section 11.2, Sample Size Determination.

4.1. Inclusion Criteria

Each potential subject must satisfy all of the following criteria to be enrolled in the study.

1. Subject must be able to read and provide consent after completing the informed consent process.

2. Subject must be a man or woman aged ≥18 to ≤50 years.
3. Subject must be healthy on the basis of physical examination, medical history, and the investigator's clinical judgment.
4. Subject must meet the following laboratory criteria within 28 days before Day 1 in the main study and within 56 days before Day 1 in the substudy*:
   - Hemoglobin: women: ≥11.5 g/dL; men ≥12.5 g/dL

     Note: based on the local laboratory's normal reference ranges.
   - White blood cell count: 3,501 to 10,999 cells/mm^3, inclusive
   - Platelets: 130,001 to 550,000 per mm^3, inclusive
   - Urinalysis (clean urine sample): protein and blood <1+, glucose negative

     Note: for women: in case of menstruation, urinalysis must be postponed but a result should be available before Day 1.
   - Alanine aminotransferase/aspartate aminotransferase (ALT/AST) ≤1 x institutional upper limit of normal
   - Serum creatinine ≤1 x institutional upper limit of normal
   - Troponin I ≤1 x institutional upper limit of normal
   - Fibrinogen ≤1 x institutional upper limit of normal
   - Prothrombin time ≤1 x institutional upper limit of normal
   - Activated partial thromboplastin time ≤1 x institutional upper limit of normal.

   * If laboratory screening tests are out of range, repeat of screening tests is permitted once, provided there is an alternative explanation for the out of range value.
5. All women of childbearing potential must:
   - Have a negative serum β-human chorionic gonadotropin (β-hCG) pregnancy test at screening
   - Have a negative urine β-hCG pregnancy test immediately prior to each study vaccine administration
   - Practice adequate birth control measures from 28 days before the prime vaccination until at least 3 months after the boost vaccination (see also Section 9.1.3, Screening). The following birth control measures will be considered adequate:
     - Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, and transdermal); progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, and implantable); intrauterine devices (IUD) and intrauterine hormone-releasing systems (IUS); vasectomized partner
     - Abstinence (defined as refraining from heterosexual intercourse during participation in the study [from 28 days before the prime vaccination until at least 3 months after the boost vaccination])
   - If not heterosexually active at screening, must agree to practice adequate birth control

measures if they become heterosexually active during participation in the study (from the start of screening onwards until at least 3 months after the boost vaccination).

– Agree not to donate eggs (ova, oocytes) for the purposes of assisted reproduction from the start of screening onwards until at least 3 months after the boost vaccination.

6. Women of non-childbearing potential, defined as postmenopausal (>45 years of age with amenorrhea for ≥2 years or any age with amenorrhea for ≥6 months and serum follicle-stimulating hormone [FSH] >40 mIU/mL) or surgically sterile (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy), are not required to use the birth control methods as described in Inclusion Criterion #5.
7. A man who has not had a vasectomy and is sexually active with a woman of childbearing potential must use a double-barrier method of birth control, such as either condom with spermicidal foam/gel/film/cream/suppository or partner with occlusive cap (diaphragm, cervical/vault caps) with spermicidal foam/gel/film/cream/suppository. In case the female partner is using an adequate method of birth control (see Inclusion Criterion #5), a single-barrier method of birth control for the male subject is acceptable. Men must also agree not to donate sperm from the start of screening onwards until at least 3 months after the boost vaccination.
8. Subject must be available and willing to participate for the duration of the study visits and follow-up.
9. Subject must be willing to provide verifiable identification.
10. Subject must be willing to provide his/her National Insurance/Passport number for the purpose of The Over-volunteering Prevention System (TOPS) registration.
11. Subject must have a means to be contacted.

4.2. Exclusion Criteria

Any potential subject who meets any of the following criteria will be excluded from participating in the study.

1. Has been vaccinated with a candidate Ebola vaccine.
2. Has been diagnosed with Ebola disease or exposed to Ebola including travel to West Africa in the last 12 months. West Africa includes but is not limited to the countries of Guinea, Liberia, Mali, and Sierra Leone.
3. Has received any Ad26- or MVA-based candidate vaccine in the past.
4. Known allergy or history of anaphylaxis or other serious adverse reactions to vaccines or vaccine products (including any of the constituents of the study vaccines [eg, polysorbate 80, ethylenediaminetetraacetic acid (EDTA) or L-histidine for Ad26.ZEBOV vaccine; and tris (hydroxymethyl)-amino methane (THAM) for MVA-BN-Filo vaccine]), including known allergy to egg, egg products and aminoglycosides.
5. Subjects with acute illness (this does not include minor illnesses such as diarrhea or mild upper respiratory tract infection) or temperature ≥38.0ºC on Day 1 will be excluded from enrollment into the study.

6. Chronic active hepatitis B or hepatitis C infection, documented by hepatitis B surface antigen (HBsAg) and hepatitis C antibody, respectively.
7. HIV type 1 or type 2 infection.
8. A woman who is pregnant or breast-feeding, or planning to become pregnant while enrolled in the study or within 3 months after the boost vaccination.
9. Bleeding or clotting disorders.
10. Any clinically significant acute or chronic medical condition that, in the opinion of the investigator, would preclude participation (eg, history of seizure disorders, autoimmune disease, any spleen disease, active malignancy, active tuberculosis, asthma, other systemic infections, abnormal laboratory safety parameters*).

    * Grade 1 abnormalities for laboratory tests other than those covered in Inclusion Criterion #4 are not an exclusion criterion. If laboratory tests other than those covered in Inclusion Criterion #4 are out of range (grade 2 or grade 3), repeat of screening tests is permitted once, provided there is an alternative explanation for the out of range value.
11. History of malignancy other than squamous cell or basal cell skin cancer, unless there has been surgical excision that is considered to have achieved cure. Subjects with a history of skin cancer must not be vaccinated at the previous tumor site.
12. Post-organ and/or stem cell transplant whether or not with chronic immunosuppressive therapy.
13. Major surgery (per the investigator's judgment) within the 4 weeks prior to study entry or planned major surgery through the course of the study.
14. History of myocarditis, pericarditis, cardiomyopathy, transient ischemic attack or stroke, myocardial infarction, angina, coronary artery disease, congestive heart failure, clinically significant arrhythmia (including any arrhythmia requiring medication, treatment, or clinical follow-up).
15. Electrocardiogram (ECG) with clinically significant findings, or features that would interfere with the assessment of myocarditis/pericarditis, including any of the following:
    - Conduction disturbance (complete left or complete right bundle branch block or nonspecific intraventricular conduction disturbance with QRS ≥120 ms, PR interval ≥210 ms, any second- or third-degree atrioventricular block, or prolongation of the QT interval corrected according to Fridericia's formula [QTcF] [>450 ms])
    - Significant repolarization (ST-segment or T-wave) abnormality.
    - Significant atrial or ventricular arrhythmia; frequent atrial or ventricular ectopy (eg, frequent premature atrial contractions, 2 premature ventricular contractions in a row).
    - ST-elevation consistent with ischemia or evidence of past or evolving myocardial infarction.
16. History of diabetes mellitus type 1 or type 2, including cases controlled with diet alone.

    Note: history of isolated gestational diabetes is not an exclusion criterion.
17. Thyroidectomy, or thyroid disease requiring medication during the last 12 months.

18. Uncontrolled hypertension, defined as systolic blood pressure ≥140 mmHg at enrollment or diastolic blood pressure ≥90 mmHg at enrollment.

    Note: In case of diastolic values greater than 90 mmHg or systolic values greater than 140 mmHg, the measurement can be repeated after 30 minutes rest and the subject can be enrolled in case of 2 consecutive measurements fulfilling the inclusion criterion.

19. Major psychiatric illness and/or substance abuse problems during the past 12 months that in the opinion of the investigator would preclude participation.
20. Receipt of live attenuated vaccines from 30 days before Day 1 or receipt of any other vaccine in the period from 15 days before Day 1.
21. Use of experimental therapeutic agents within 3 months from the start of screening.
22. Current or planned participation in another clinical study during the study period.

    Note: participation in an observational clinical study is allowed.

23. Receipt of blood products or immunoglobulin in the past 3 months.
24. Donation of a unit of blood within 8 weeks before Day 1 or plans to donate blood during participation in the study (from the start of screening onwards).
25. Current or past abuse of recreational or narcotic drugs, which in the investigator's opinion would compromise the subject's safety and/or compliance with the study procedures.

    Note: urine will be tested to check for current use of amphetamines, benzodiazepines, cocaine, cannabinoids, and opioids.

26. Current alcohol use judged by the investigator to potentially interfere with subject study adherence.
27. History of chronic urticaria (recurrent hives).
28. Chronic or recurrent use of medications which modify host immune response, eg, cancer chemotherapeutic agents, parenteral corticosteroids.
29. Subject cannot communicate reliably with the investigator.
30. Subject who, in the opinion of the investigator, is unlikely to adhere to the requirements of the study.
31. Study site employees and family members of the investigator.

NOTE: The investigator should ensure that all study enrollment criteria have been met at the end of the screening period. If a subject's status changes (including laboratory results or the receipt of additional medical records) after screening but before Day 1 such that the subject no longer meets all eligibility criteria, then the subject should be excluded from participation in the study. Section 17.4, Source Documentation, describes the required documentation to support meeting the enrollment criteria.

4.3. Prohibitions and Restrictions

Potential subjects must be willing and able to adhere to the following prohibitions and restrictions during the course of the study to be eligible for participation:

1. Traveling to epidemic Ebola areas is prohibited until the start of the long-term follow-up (see Section 4.2, Exclusion Criteria). Subjects travelling to epidemic Ebola areas will be excluded from follow-up collection of blood for immune analyses if they contract Ebola disease.
2. Subjects must use adequate birth control measures as described in Section 4.1, Inclusion Criteria.*
3. Women of childbearing potential must agree not to donate eggs (ova, oocytes) for the purposes of assisted reproduction from the start of screening onwards until at least 3 months after the boost vaccination (see Section 4.1, Inclusion Criteria).*
4. Men must agree not to donate sperm from the start of screening onwards until at least 3 months after the boost vaccination (see Section 4.1, Inclusion Criteria).*

   ** After the main study is unblinded, placebo subjects may stop birth control measures or resume their pre-study birth control measures, and may donate eggs (females) or sperm (males).*
5. Receipt of live attenuated vaccines is prohibited until 30 days after the boost vaccination, and receipt of any other vaccine is prohibited until 15 days after the boost vaccination. Medically indicated vaccines (eg, influenza [except live attenuated influenza vaccine], tetanus, hepatitis A, hepatitis B, rabies) are not prohibited, but should be given at least 15 days before (or at least 15 days after) administration of study vaccine in order to avoid potential confusion of adverse reactions. If a vaccine is indicated in a post-exposure setting (eg, rabies or tetanus), it must take priority over the study vaccine (see Section 8, Prestudy and Concomitant Therapy).

5. TREATMENT ALLOCATION AND BLINDING

Vaccination Schedule Allocation (Main Study Only)

Central randomization will be implemented in the main study. Subjects will be randomly assigned to 1 of 4 groups, and within groups randomly assigned to active vaccine or placebo, based on a computer-generated randomization schedule prepared before the study by or under the supervision of the sponsor. The randomization within each group will be balanced by using randomly permuted blocks. The interactive web response system (IWRS) will assign a unique code, which will dictate the assignment and matching study vaccine for the subject. The requestor must use his or her own user identification and personal identification number when contacting the IWRS, and will then give the relevant subject details to uniquely identify the subject.

All subjects in the **Substudy** (Group 5) will be assigned to active vaccine.

Blinding (Main Study Only)

Subjects, clinical staff and site personnel in the main study will be blinded to the study vaccine allocation within groups until the start of the long-term follow-up period, except for the pharmacist or qualified staff member with primary responsibility for study vaccine preparation and dispensing and an independent study vaccine administrator (see Definitions of Terms).

Up to the 7-day post-prime interim analysis all sponsor personnel will be blinded. Thereafter, the sponsor, except personnel involved in the statistical analysis of the 7-day post-prime data and the sponsor committee involved in making future decisions for the program will be blinded to study vaccine allocation until the primary analysis, ie, when all subjects have had their 21-day post-boost visit (Day 50 for Groups 1 and 3; Day 78 for Groups 2 and 4) or discontinued earlier. At that time, the study will be unblinded and subjects who received active vaccine will be followed up in an open-label fashion until Day 360. The sponsor committee will be unblinded upon completion of the interim analyses.

The investigator will not be provided with randomization codes. The codes will be maintained within the IWRS, which has the functionality to allow the investigator to break the blind for an individual subject.

Under normal circumstances, the blind must not be broken until the primary analysis. Otherwise, the blind should be broken only if specific emergency treatment/course of action would be dictated by knowing the treatment status of the subject. In such cases, the investigator may in an emergency determine the identity of the study vaccine. It is recommended that the investigator contact the sponsor or its designee if possible to discuss the particular situation, before breaking the blind. Telephone contact with the sponsor or its designee will be available 24 hours per day, 7 days per week. In the event the blind is broken, the sponsor must be informed as soon as possible. The date and reason for the unblinding must be documented by the IWRS, in the appropriate section of the case report form (CRF), and in the source document. The documentation received from the IWRS indicating the code break must be retained with the subject's source documents in a secure manner. If the randomization code is broken by the investigator or the study-site personnel, the subject must be withdrawn from the study and must be followed as appropriate. If the code is broken by the sponsor for safety reporting purposes, the subject may remain in the study.

6. DOSAGE AND ADMINISTRATION

An overview of the vaccination schedules is provided in Table 7 (Section 3.1, Overview of Study Design).

All subjects in the main study will receive a vaccination, according to randomization, on Days 1 and 29 (Groups 1 and 3) or on Days 1 and 57 (Groups 2 and 4) at the following dose levels:

- MVA-BN-Filo added to a diluent consisting of tris buffered saline: $1x10^8$ $TCID_{50}$, 0.5 mL
- Ad26.ZEBOV: $5x10^{10}$ vp, 0.5 mL
- Placebo: 0.9% saline, 0.5 mL

Subjects in the substudy (Group 5) will receive active vaccine on Days 1 and 15 at the same dose levels as in the main study.

MVA-BN-Filo and Ad26.ZEBOV, or placebo (main study only), will be administered as IM injections, in either deltoid in the upper arm. When choosing an arm for the injection, the study

vaccine administrator should consider whether there is an arm injury, local skin problem, or significant tattoo that precludes administering the injection or will interfere with evaluating the arm after injection. In each subject, boost vaccination should be administered in the opposite deltoid from prime vaccination and it should be recorded in the CRF in which arm the vaccination has been administered. No local or topical anesthetic will be used prior to the injection.

The Site Investigational Product Procedures Manual specifies the maximum time that will be allowed between preparation and administration of the study vaccine.

7. TREATMENT COMPLIANCE

All study vaccine will be administered by an independent study vaccine administrator (see Definitions of Terms). The date and time of each study vaccine administration will be recorded in the CRF.

8. PRESTUDY AND CONCOMITANT THERAPY

Prestudy therapies up to 30 days prior to the start of screening must be recorded in the CRF.

Concomitant therapies must be recorded from the prime vaccination onwards until the start of the long-term follow-up period. Concomitant therapies should also be recorded during the long-term follow-up but only if given in conjunction with serious adverse events that meet the criteria outlined in Section 12.3.2, Serious Adverse Events.

Vaccination with live attenuated vaccines in the period from 30 days before Day 1 to 30 days after the boost vaccination, and vaccination with any other vaccine in the period from 15 days before Day 1 to 15 days after the boost vaccination, are prohibited (per Exclusion Criterion #20 and Prohibitions and Restrictions Criterion #5). Medically indicated vaccines (eg, influenza [except live attenuated influenza vaccine], tetanus, hepatitis A, hepatitis B, or rabies) are not prohibited, but should be given at least 15 days before (or at least 15 days after) administration of any study vaccine to avoid potential confusion of adverse reactions. However, if a vaccine is indicated in the post-exposure setting (eg, rabies or tetanus), it must take priority over the study vaccine.

Study subjects can receive medications, such as paracetamol, non-steroidal anti-inflammatory drugs or antihistamines as needed. Use of these medications as routine prophylaxis prior to study vaccine administration is prohibited. Chronic or recurrent use of medications that modify the host immune response (eg, parenteral corticosteroids, cancer chemotherapeutic agents) are prohibited (per Exclusion Criterion #28).

Subjects must use adequate birth control measures as described in Section 4.1, Inclusion Criteria.

The sponsor must be notified in advance (or as soon as possible thereafter) of any instances in which prohibited therapies are administered.

9. STUDY EVALUATIONS

9.1. Study Procedures

9.1.1. Overview

The Time and Events Schedule summarizes the frequency and timing of safety, tolerability and immunogenicity assessments applicable to this study. Additional study visits may be required if in the investigator's opinion, further clinical or laboratory evaluation is needed.

Additional serum or urine pregnancy tests may be performed, as determined necessary by the investigator or required by local regulation, to establish the absence of pregnancy at any time during the subject's participation in the study.

From screening onwards until the start of the long-term follow-up period, the total blood volume to be collected from each subject will be approximately 505 mL (Groups 1, 3 and 5), or 565 mL (Groups 2 and 4). The total blood volume to be collected from each subject enrolled into the long-term follow-up will be approximately 180 mL.

Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

9.1.2. Visit Windows

Visit windows that will be allowed are summarized in Table 8. If a subject did not receive study vaccine on the planned day of vaccination, the timings of the next visits post vaccination (see Time and Events Schedule) will be determined relative to the actual day of vaccination.

Table 8: Visit Windows

Visit Description	Day	Window
Boost Vaccination	Day 15 for Group 5; Day 29 for Groups 1 and 3; Day 57 for Groups 2 and 4	±1 day
Three Days Post Vaccination (Prime and Boost)	Days 4 and 18 for Group 5; Days 4 and 32 for Groups 1 and 3; Days 4 and 60 for Groups 2 and 4	±1 day
Seven Days Post Vaccination (Prime and Boost)	Days 8 and 22 for Group 5; Days 8 and 36 for Groups 1 and 3; Days 8 and 64 for Groups 2 and 4	±1 day
Twenty-eight Days Post Vaccination (Prime) (Groups 2 and 4 Only)	Day 29	±2 days
Twenty-one Days Post Vaccination (Boost)	Day 36 for Group 5; Day 50 for Groups 1 and 3; Day 78 for Groups 2 and 4	±3 days
Follow-up	Day 180 for all groups	±15 days
	Day 240 for all groups	±30 days
	Day 360 for all groups	±30 days

9.1.3. Screening

Up to 28 days (main study) or up to 56 days (substudy) before the baseline visit (Day 1; day of prime vaccination), screening assessments as indicated in the Time and Events Schedule will occur. Screening may be split into multiple days or visits.

The ICF will be signed before any study-specific procedures at the start of the screening period (see Section 16.2.3, Informed Consent). For men and for women of non-childbearing potential (defined in Inclusion Criterion #6) there will be no minimum duration of the screening period and it will last only for the time required to verify eligibility criteria. For women of childbearing potential, it should be confirmed that adequate birth control measures were used from at least 28 days before the prime vaccination with a negative serum β-hCG pregnancy test available before Day 1 and a negative urine test immediately prior to each study vaccination. All men and women, except for women of non-childbearing potential, will be asked to use adequate birth control for sexual intercourse from signing of the ICF onwards until at least 3 months after the boost vaccination.

Only healthy subjects complying with the criteria specified in Section 4, Subject Population, will be included in the study. The investigator will provide detailed information on the study to the subject and will obtain written informed consent prior to each subject's study participation. The procedures indicated in the Time and Events Schedule will only be performed after the subject's written informed consent has been obtained. Screening may be conducted in part via a non-study specific screening consent process; however, no study-specific procedures should take place until the subject has signed the study-specific ICF.

The following is performed to determine the eligibility requirements as specified in the inclusion and exclusion criteria:

- Review of all inclusion and exclusion criteria
- Review of medical history and demographics
- Review of prestudy therapies
- Serum pregnancy test (for women of childbearing potential)
- Blood sampling for hematology and chemistry (fasting or non-fasting)
- Urine sampling for urinalysis
- Serology testing (HIV type 1 or type 2, hepatitis B, hepatitis C)
- FSH assessment (for women >45 years of age who are postmenopausal for less than 2 years or at any age with amenorrhea for more than 6 months)
- Urine drug screen
- Full physical examination
- Measurement of vital signs (heart rate, blood pressure, oral body temperature)

- 12-lead ECG

The overall eligibility of the subject to participate in the study will be assessed once all screening values and results of any other required evaluations are available. Retesting of values (eg, safety laboratory) that lead to exclusion is allowed once using an unscheduled visit during the screening period to assess eligibility, provided there is an alternative explanation for the out of range value. Study subjects who qualify for inclusion will be contacted and scheduled for enrollment and prime vaccination within 28 days (main study) or 56 days (substudy).

All adverse events will be collected from signing of the ICF onwards until 21 days post-boost (Day 36 for Group 5, Day 50 for Groups 1 and 3, and Day 78 for Groups 2 and 4), and serious adverse events and adverse events related to blood draws will be collected from signing of the ICF onwards until the end of the study.

9.1.4. Vaccination

Prime Vaccination – Day 1

The investigator should ensure that all enrollment criteria have been met during screening. If a subject's status (including any unscheduled laboratory results or the receipt of additional medical records) after screening but before the prime vaccination changes such that the subject no longer meets all enrollment criteria, then the subject should be excluded from participation in the study. If the initial laboratory sampling occurred more than 28 days (main study) or 56 days (substudy) before baseline (Day 1), sampling will need to be repeated.

Following a re-check of the inclusion and exclusion criteria, a urine pregnancy test (for women of childbearing potential), a physical examination, and measurements of vital signs, eligible subjects will be allocated to a vaccination schedule as described in Section 5, Treatment Allocation and Blinding, and receive treatment as described in Section 6, Dosage and Administration, unless any of the pre-specified criteria not to proceed with vaccination are met (for details, see Section 10.2, Discontinuation of Study Vaccine, Section 10.3, Contraindications to Vaccination, Section 11.8, Pausing Rules).

Pre-vaccination samples for hematology, chemistry and urinalysis will be collected (for details, see Section 9.3.2). In addition, before the prime vaccination, subjects will have blood drawn for immunologic assays.

Study vaccine will be prepared on-site by an unblinded pharmacist or qualified staff member (see Section 14.3 for details) who will send the vaccine to an independent study vaccine administrator (see Definitions of Terms) for administration to the subject. To preserve blinding (in the main study only), the pharmacist or qualified staff member will place a blinding tape on the syringe to mask its content. After vaccination, subjects will be directly observed for 30 minutes for reactogenicity (to record local and systemic adverse events) and vital signs measurements will be repeated at 30 and 60 minutes post-vaccination. Subjects will remain at the site for a total of 60 minutes post-vaccination to monitor for development of any acute reactions, or longer in case of grade 3 adverse events.

Upon discharge from the site, subjects will be instructed to contact immediately the investigator if they experience any adverse event that they perceive as relevant or possibly related to study vaccine in their opinion. Subjects will be contacted after at least 24 hours for collection of adverse events and to confirm that no pre-specified pausing rules have been met.

Subjects will be provided with a diary, a thermometer, and a ruler to measure and record local solicited adverse events and body temperature. Subjects will also record symptoms of unsolicited and solicited local and systemic adverse events in the diary in the evening after each vaccination and then daily for the next 7 days. Subjects will be instructed to contact the investigator in case they experience severe and/or serious adverse events.

All adverse events and serious adverse events will be collected and documented on the CRFs, together with the information on any concomitant medications.

Boost Vaccination – Day 15 in Group 5, Day 29 in Groups 1 and 3; Day 57 in Groups 2 and 4

Subjects will receive the boost vaccination, unless any of the pre-specified criteria not to proceed with vaccination are met (see Section 10.2, Discontinuation of Study Vaccine, Section 10.3, Contraindications to Vaccination, Section 11.8, Pausing Rules)

A urine pregnancy test (women of childbearing potential), a physical examination, and vital sign measurements will be performed before study vaccine administration. Pre-vaccination samples for hematology, chemistry and urinalysis will also be collected (for details, see Section 9.3.2). In menstruating women, urinalysis will be postponed to the next visit. All subjects will also have blood drawn for immunologic assays.

Study vaccine will be prepared on-site by an unblinded pharmacist or qualified staff member (see Section 14.3 for details) who will send the vaccine to an independent study vaccine administrator (see Definitions of Terms) for administration to the subject. To preserve blinding (in the main study only), the pharmacist or qualified staff member will place a blinding tape on the syringe to mask its content. After vaccination, subjects will be directly observed for 30 minutes for reactogenicity (to record local and systemic adverse events) and vital signs measurements will be repeated at 30 and 60 minutes post-vaccination. Subjects will remain at the site for a total of 60 minutes post-vaccination to monitor for development of any acute reactions, or longer in case of grade 3 adverse events.

Upon discharge from the site, subjects will be instructed to contact immediately the investigator if they experience any adverse event that they perceive as relevant or possibly related to study vaccine in their opinion. Subjects will be contacted after at least 24 hours for collection of adverse events and to confirm that no pre-specified pausing rules have been met.

Subjects will be provided with a diary, a thermometer, and a ruler to measure and record local solicited adverse events and body temperature. Subjects will also record symptoms of unsolicited and solicited local and systemic adverse events in the diary in the evening after each vaccination

and then daily for the next 7 days. Subjects will be instructed to contact the investigator in case they experience severe and/or serious adverse events.

All adverse events and serious adverse events will be collected and documented on the CRFs, together with the information on any concomitant medications.

9.1.5. Post Vaccination

In all groups, subjects will come to the clinic at 3, 7 and 21 days post vaccination as described below and indicated in the Time and Events Schedule. Subjects in Groups 2 and 4 will also have to come to the site at 28 days after the prime vaccination (Day 29) for collection of blood for additional immunologic assays. End-of-study assessments will be performed in blinded condition on the 21-day post-boost visit (for subjects who received placebo in the main study) or on the Day 360 visit (for subjects who received active vaccine). After unblinding, subjects who received placebo in the main study will be contacted to communicate that they have completed the study and do not need to contact the site any longer.

Adverse events will be collected at all visits until 21 days post-boost (Day 36 for Group 5, Day 50 for Groups 1 and 3; Day 78 for Groups 2 and 4), and serious adverse events and adverse events related to blood draws will be collected at all visits until the end of the study. All aforementioned events will be documented on the CRFs, together with the information on any concomitant medications at the specified visits in the Time and Events Schedule.

Three Days Post Vaccination (Prime and Boost) Visit – Days 4 and 18 for Group 5; Days 4 and 32 for Groups 1 and 3; Days 4 and 60 for Groups 2 and 4

Samples will be collected for hematology and chemistry (see Section 9.3.2 for details), an ECG will be performed, and the investigator or designee will review information from the subject's diary in order to complete the relevant parts of the CRF.

Seven Days Post Vaccination (Prime and Boost) Visit – Days 8 and 22 for Group 5; Days 8 and 36 for Groups 1 and 3; Days 8 and 64 for Groups 2 and 4

Samples will be collected for hematology, chemistry and urinalysis (see Section 9.3.2 for details), and blood will be drawn for immunologic assays. The investigator or the designee will review information from the subjects' diary in order to complete the relevant parts of the CRF.

Twenty-eight Days Post Vaccination (Prime) Visit (Groups 2 and 4 Only) – Day 29

Subjects in Groups 2 and 4 will have blood drawn for immunologic assays at 28 days after the prime vaccination.

Twenty-one Days Post Vaccination (Boost) Visit – Day 36 for Group 5; Day 50 for Groups 1 and 3; Day 78 for Groups 2 and 4

A physical examination will be performed, and blood will be drawn for immunologic assays. Subjects will be instructed to contact the investigator if they experience any adverse event or

intercurrent illness that they perceive as relevant and/or can be possibly related to study vaccine in their opinion.

9.1.6. Long-term Follow-up

When all subjects have had their 21-day post-boost visit (Day 36 for Group 5; Day 50 for Groups 1 and 3, and Day 78 for Groups 2 and 4), or discontinued earlier, all subjects who received active vaccine will enter the long-term follow-up, with visits on Days 180 (±15 days), 240 (±30 days) and 360 (±30 days) post-prime. At each of these 3 visits, blood will be drawn for immunologic assays, and serious adverse event information and adverse events related to blood draws will be collected.

9.1.7. VAC52150 Vaccine Development Registry

Subjects enrolled in this study will be approached to consent for enrollment into a product development registry protocol (to be developed) for long-term safety surveillance. The primary objective of the registry is to collect cumulative serious adverse event information and pregnancy outcomes from all subjects who have entered the first VAC52150 clinical development study (or another eligible study) up to 4 years after the end of the present study.

9.1.8. Early Withdrawal

In case of early withdrawal due to an adverse event, the investigator will collect all information relevant to the adverse event and safety of the subject, and will follow the subject until resolution or until reaching a clinically stable endpoint. If feasible, blood will be drawn for immunologic assays. Subjects who wish to withdraw consent will be offered an optional visit for safety follow-up (before the formal withdrawal of consent). The subject has the right to refuse.

For more information, see Section 10.4, Withdrawal From the Study.

9.2. Immunogenicity

9.2.1. Endpoints

Immune Responses (Secondary Objectives):

Analysis of the immunogenicity of the study vaccine regimens will include the characterization of antibodies as well as specific cellular responses.

Serology

- Virus neutralization assay: neutralizing antibody reactivity against the EBOV GP will be defined as the serum titer that is able to inhibit viral infection by a certain percentage.
- EBOV GP protein ELISA: antibody levels against the EBOV GP will be determined to identify the binding capacity of the antibodies elicited.

Cell-mediated immune response

- IFN-γ ELISpot assays: the presence and functional capacity of T-cells will be determined after pathogen-specific stimulation of peripheral blood mononuclear cells (PBMCs) with EBOV

GP-specific peptides. Cytokine-producing T-cells can be quantified using ELISpot technology.

Immune Responses (Exploratory Objectives):

Additional exploratory analyses may be performed to further investigate study vaccine-elicited immune responses. These may include, but will not be limited to, the following assays:

Serology

- Adenovirus and MVA neutralization assays: to assess neutralizing antibody responses against the Ad26 or MVA vector.
- Humoral responses to different EBOV GPs, the SUDV GP, MARV GP and TAFV NP if assays are available.
- Molecular antibody characterization: molecular characterization of study vaccine-elicited antibodies may include, but will not be limited to, Fc characterization, isotype analysis and epitope mapping.

Cell-mediated immune response

- ICS: activation of CD4 and CD8 T-cell subsets and their cytokine expression patterns may be determined by flow cytometry after EBOV GP-specific stimulation (including, but not limited to, IFN-γ, interleukin [IL]-2, and tumor necrosis factor [TNF]-α). Exploratory phenotypic and functional analysis may be included.
- Cellular responses to the SUDV GP, MARV GP and TAFV NP if assays are available.

9.2.2. Evaluations

Venous blood samples will be collected for the determination of immune responses at the time points and in volumes as indicated in the Time and Events Schedule.

The immunologic assays and purposes are summarized in Table 9 and Table 10. The exploratory assay package may include, but will not be limited to, the listed assays. Sample collection and processing will be performed by the site staff according to current versions of approved standard operating procedures. The Laboratory Manual contains further details regarding the collection, handling, labeling, and shipment of blood samples to the respective laboratories.

Table 9: Summary of Immunologic Assays (Serology)

Assay	Purpose
Secondary endpoints	
Virus neutralization assay	Analysis of neutralizing antibodies to EBOV GP
ELISA	Analysis of antibodies binding to EBOV GP
Exploratory endpoints	
Adenovirus/MVA neutralization assay	Neutralizing antibodies to adenovirus/MVA
Molecular antibody characterization	Analysis of anti-EBOV GP, SUDV GP, MARV GP and/or TAFV NP antibody characteristics, including IgG subtyping
Exploratory ELISA	Analysis of binding antibodies to a different source of EBOV GP

EBOV: Ebola virus; ELISA: enzyme-linked immunosorbent assay; GP: glycoprotein; IgG: immunoglobulin G; MARV: Marburg virus; MVA: Modified Vaccinia Ankara; NP: nucleoprotein; SUDV: Sudan virus; TAFV: Tai Forest virus

Table 10: Summary of Immunologic Assays (Cellular)

Assay	Purpose
Secondary endpoints	
ELISpot	T-cell IFN-γ responses to EBOV GP
Exploratory endpoints	
ICS and/or ELISpot of frozen PBMC	Analysis of T-cell responses to EBOV GP, SUDV GP, MARV GP and/or TAFV NP (including CD4/8, IL-2, IFN-γ, TNF-α and/or activation markers)
ICS of fresh PBMC	Analysis of T cell responses to EBOV GP including CD4-positive and low-magnitude T cell responses

EBOV: Ebola virus; ELISpot: enzyme-linked immunospot; GP: glycoprotein; ICS: intracellular cytokine staining; IFN: interferon; IL: interleukin; MARV: Marburg virus; NP: nucleoprotein; PBMC: peripheral blood mononuclear cells; SUDV: Sudan virus; TAFV: Tai Forest virus; TNF: tumor necrosis factor

Future scientific research may be conducted to further investigate Ebola vaccine- and disease-related questions. This may include the development of new or the improvement of existing techniques to characterize EBOV-directed immune responses or diagnostic tests. No additional samples will be taken for these analyses, however residual samples from other tests (eg, plasma from safety specimens) may be retained for these purposes.

9.3. Safety

9.3.1. Endpoints

The safety and tolerability endpoints are:

- Adverse events, collected from signing of the ICF onwards until 21 days post-boost (Day 36 for Group 5, Day 50 for Groups 1 and 3, and Day 78 for Groups 2 and 4)
- Serious adverse events and adverse events related to blood draws, collected from signing of the ICF onwards until the end of the study
- Solicited local and systemic adverse events (reactogenicity), collected until 7 days after each study vaccine administration

9.3.2. Evaluations

Any clinically relevant safety-related changes that occur during the study must be recorded on the CRF. Any clinically significant abnormalities persisting at the end of the study or upon early withdrawal will be followed by the investigator until resolution or until a clinically stable endpoint is reached.

The study will include the following evaluations of safety and tolerability according to the time points provided in the Time and Events Schedule:

Adverse Events

Adverse events will be collected at all visits from signing of the ICF onwards until 21 days post-boost. Serious adverse events and adverse events related to blood draws will be collected from signing of the ICF onwards until the end of the study. Reactogenicity (solicited local and systemic adverse events; see below) will be reported by the subject until 7 days after each administration of study vaccine.

Adverse events and serious adverse events will be followed by the investigator as specified in Section 12, Adverse Event Reporting.

Solicited Adverse Events

Solicited adverse events are precisely defined events that subjects are specifically asked about and which are noted by subjects in the diary. The investigator or the designee should discuss the information from the diary with the subject, document the relevant information in the clinic chart. Solicited adverse events will be captured on a separate CRF page as described in the CRF Completion Guidelines.

Solicited Local (Injection Site) Reactions

Subjects will also be instructed on how to note occurrences of erythema, induration and swelling (measured using the ruler supplied), and pain/tenderness, itching, and/or warmth at the injection site daily until 7 days after each administration of study vaccine. These occurrences will be recorded in the diary provided to serve as a reminder to the subject for the next visit.

Solicited Systemic Adverse Events

Subjects will be instructed on how to record daily temperature using a thermometer provided for home use. Subjects should record the oral temperature in the evening post vaccination, and then daily for the next 7 days in the diary. Temperature should be measured at approximately the same time each day. If more than one measurement is made on any given day, the highest temperature will be recorded in the CRF.

If a solicited local or systemic adverse event is not resolved at Day 8, the follow-up will be captured on the diary. The subject will be instructed to record the date of last symptoms and maximum severity in the diary after resolution.

Subjects will also be instructed on how to note the following symptoms in the diary until 7 days after each vaccination:

- fatigue
- headache
- myalgia
- arthralgia
- chills
- nausea
- vomiting
- rash
- general itching

Clinical Laboratory Tests

Samples will be collected for hematology, serum chemistry and urinalysis. The investigator must review the laboratory report, document this review, and record any clinically relevant changes on the adverse event page of the CRF. Laboratory reports must be filed with the source documents.

The following tests will be performed by the local laboratory at the time points indicated in the Time and Events Schedule, unless otherwise specified. Parameters marked with an asterisk (*) will only be measured at screening.

- Hematology and Coagulation Panel

  -hemoglobin
  -hematocrit
  -red blood cell count
  -white blood cell count with differential
  -platelet count
  -erythrocyte sedimentation rate (ESR)
  -C-reactive protein (CRP)
  -fibrinogen*
  -prothrombin time
  -activated partial thromboplastin time

- Serum Chemistry Panel

  -sodium
  -potassium
  -chloride*
  -calcium*
  -magnesium*
  -bicarbonate*
  -blood urea nitrogen (BUN)
  -alkaline phosphatase*
  -AST
  -ALT
  -glucose (fasting or non-fasting)*
  -phosphate*
  -albumin*
  -total protein*
  -total bilirubin*
  -creatinine
  -troponin I*
  -FSH* (only in women >45 years of age who are postmenopausal <2 years or at any age with amenorrhea >6 months)

- Urinalysis - Dipstick

  -specific gravity
  -pH
  -glucose
  -protein
  -blood
  -ketones

Microscopic examination will be carried out in the event of positive urinalysis dipstick tests.

The laboratory values will be determined according to the DMID Toxicity Table for Use in Trials Enrolling Healthy Adults (2014) (see Attachment 1) if applicable for laboratory tests, and, if clinically significant per investigator, reported as adverse events.

Additional clinical laboratory assessments to be performed are as follows:

- Serum pregnancy test for women of childbearing potential at screening
- Urine pregnancy test for women of childbearing potential before each study vaccination
- Serology (HIV type 1 and type 2, hepatitis B, hepatitis C) at screening
- Urine drug screen at screening

Electrocardiogram (ECG)

A single, 12-lead ECG will be performed at screening and at 3 days after each study vaccination. The ECGs will be read locally. ECGs may be repeated at other time points during the study if clinically indicated based on signs and symptoms.

During the collection of an ECG, subjects should be in a quiet setting without distractions (eg, television, cell phones). Subjects should rest in a supine position for at least 5 minutes before ECG collection and should refrain from talking or moving arms or legs. If blood sampling or vital sign measurement is scheduled for the same time point as ECG recording, the procedures should be performed in the following order: ECG(s), vital signs, blood draw.

Any clinically relevant abnormalities occurring from signing of the ICF onwards until 21 days post-boost must be recorded on the adverse event page of the CRF. Thereafter, recording will be limited to serious adverse events. All events should be followed to resolution, or until reaching a clinically stable endpoint.

Vital Signs (oral temperature, heart rate, systolic and diastolic blood pressure)

Vital sign measurements will be performed at the time points indicated in the Time and Events Schedule. Confirmatory vital sign measurements can be performed if inconsistent with a prior measurement.

Any clinically relevant abnormalities occurring from signing of the ICF onwards until 21 days post-boost must be recorded on the adverse event page of the CRF. Thereafter, recording will be limited to serious adverse events. All events should be followed to resolution, or until reaching a clinically stable endpoint.

Physical Examination

A full physical examination, including height, body weight and calculation of the body mass index (BMI), will be carried out at screening. At other visits, an abbreviated, symptom-directed examination will be performed as indicated by the investigator based on any clinically relevant

issues, clinically relevant symptoms and medical history. The symptom-directed physical examination may be repeated if deemed necessary by the investigator.

Physical examinations will be performed by the investigator or by designated medically-trained clinician.

Any clinically relevant abnormalities occurring from signing of the ICF onwards until 21 days post-boost must be recorded on the adverse event page of the CRF. Thereafter, recording will be limited to serious adverse events. All events should be followed to resolution, or until reaching a clinically stable endpoint.

9.4. Vaccine Induced Seropositivity (VISP)

In general, uninfected subjects who participate in Ebola vaccine studies may develop Ebola-specific antibodies as a result of an immune response to the candidate Ebola vaccine, referred to as VISP. These antibodies may be detected in Ebola serologic tests, causing the test to appear positive even in the absence of actual Ebola infection. VISP may become evident during the study, or after the study has been completed.

Subjects should not donate a unit of blood within 8 weeks before Day 1 or plans to donate blood during participation in the study (from the start of screening onwards; Exclusion Criterion #24). Blood donation options for those subjects who wish to resume blood donation will be explained at the final study follow-up visit.

In the case of VISP, if, either during the study or after the end of the study, a subject requires an Ebola test outside the study (eg, to obtain a travel visa or insurance, or for medical reasons), he/she should contact the research center. The center can issue a written statement giving details on VISP.

9.5. Sample Collection and Handling

The actual dates of sample collection must be recorded in the CRF or laboratory requisition form. See the Time and Events Schedule for the timing and frequency of all sample collections.

Instructions for the collection, handling, storage, and shipment of immunogenicity samples will be provided in the Laboratory Manual. Collection, handling, storage, and shipment of samples must be under the specified, and where applicable, controlled temperature conditions as indicated in the Laboratory Manual.

10. SUBJECT COMPLETION/WITHDRAWAL

10.1. Completion

A subject will be considered to have completed the study if he/she has completed all end-of-study assessments on the 21-day post-boost visit (subjects who received placebo) or the Day 360 visit (subjects who received active vaccine).

10.2. Discontinuation of Study Vaccine

If a subject's study vaccine must be discontinued before the end of the vaccination schedule, this will not result in automatic withdrawal of the subject from the study.

Subjects will be withdrawn from study vaccine administration for the reasons listed below. These subjects must not receive any further study vaccine, but should continue to be monitored for safety and for immunogenicity if this does not result in safety risk for the subject.

- Any reason listed in Section 11.8, Pausing Rules, for preventing a subject from receiving boost vaccination
- Pregnancy
- Any adverse event considered at least possibly related to study vaccine, worsening of health status or intercurrent illnesses that, in the opinion of the investigator, requires discontinuation from study vaccine
- Confirmed Ebola disease
- Intake of disallowed medications (see Section 8, Prestudy and Concomitant Therapy)
- If the randomization code is broken by the investigator or the study-site personnel

10.3. Contraindications to Vaccination

The following events constitute a contraindication to vaccination at that point in time. If any of these events occur at the scheduled time for vaccination, the subject may be vaccinated up to 10 days beyond the window allowed for the scheduled vaccination*, or be withdrawn from that vaccination at the discretion of the investigator and after consultation with the sponsor:

- Acute illness at the time of vaccination (this does not include minor illnesses such as diarrhea or mild upper respiratory tract infection)
- Fever (oral temperature ≥38.0°C) at the time of vaccination

* In case the boost vaccination is postponed, the timing of the safety/immunogenicity visits post-boost will be planned relative to the actual vaccination day.

Note that medically indicated vaccines should be administered at least 15 days before or 15 days after study vaccine administration (see Section 8, Prestudy and Concomitant Therapy).

10.4. Withdrawal From the Study

Each subject has the right to withdraw at any time for whatever reason without affecting the right to treatment by the investigator. The investigator should make an attempt to contact subjects who did not return for scheduled visits or follow-up. Although the subject is not obliged to give reason(s) for withdrawing early, the investigator should make a reasonable effort to ascertain the reason(s) while fully respecting the subject's rights.

A subject will be withdrawn from the study for any of the following reasons:

- Repeated failure to comply with protocol requirements
- Decision by the sponsor to stop or cancel the study
- Decision by the investigator to withdraw subjects
- Decision by local regulatory authorities and Institutional Review Board/Independent Ethics Committee (IRB/IEC) to stop or cancel the study
- Lost to follow-up
- Withdrawal of consent
- Death

If a subject is lost to follow-up, every reasonable effort must be made by the study site personnel to contact the subject and to determine the reason for discontinuation/withdrawal. The measures taken for at least 3 efforts to follow-up must be documented.

When a subject withdraws before completing the study, the reason for withdrawal is to be documented in the CRF and in the source document. Study vaccine assigned to the withdrawn subject may not be assigned to another subject.

Subjects who withdraw after randomization but before the prime vaccination will be replaced. Enrollment will be stopped when 18 subjects in each group in the main study and 15 subjects in the substudy have received at least one study vaccination. Subjects who withdraw after receiving the prime vaccination will not be replaced. If a subject withdraws early from the study, early withdrawal assessments should be obtained (see Section 9.1.8, Early Withdrawal).

A subject who wishes to withdraw consent from participation in the study will be offered an optional visit for safety follow-up (before formal withdrawal of consent). The subject has the right to refuse.

Subject who withdraw from the study have the following options for optional research samples:

- The collected samples will be retained and used in accordance with the subject's original informed consent for optional research samples.
- The subject may withdraw consent for optional research samples, in which case the samples will be destroyed and no further testing will take place. To initiate the sample destruction process, the investigator must notify the sponsor study site contact about withdrawal of consent for the optional research samples and to request sample destruction. If requested, the investigator will receive written confirmation from the sponsor that the samples have been destroyed.

Withdrawal From the Optional Research Samples While Remaining in the Study

The subject may withdraw consent for optional research samples while remaining in the study. In such a case, the optional research samples will be destroyed. The sample destruction process will proceed as described above.

Withdrawal From the Use of Samples in Future Research

The subject may withdraw consent for use of samples for research (refer to Section 16.2.5, Long-Term Retention of Samples for Additional Future Research). In such a case, samples will be destroyed after they are no longer needed for the clinical study. Details of the sample retention for research are presented in the main ICF.

11. STATISTICAL METHODS

Statistical analysis will be done by the sponsor or under the authority of the sponsor. A general description of the statistical methods to be used to analyze the safety and immunogenicity data is outlined below. Specific details will be provided in the Statistical Analysis Plan.

Three interim analyses are planned in the main study for decision-making for future studies. The first interim analysis will assess safety data and will be performed when all subjects in the main study have completed the 7 days post-prime visit. The second interim analysis will be performed when all subjects in the Sentinel Cohorts and in Cohort 1 (26 subjects) have completed the 28 days post-prime visit to assess immunogenicity. The third interim analysis will be performed when all remaining subjects in Cohort 2 (46 subjects) have completed the 28 days post-prime visit to assess safety and immunogenicity. This analysis will include all available data from the main study up to this point.

Two interim analyses are planned in the substudy. The first interim analysis will assess safety data and will be performed when all subjects in Group 5 have completed the 7 days post-prime visit. The second interim analysis will be performed when all subjects have completed the 21-day post-boost visit (Day 36) or discontinued earlier. This analysis will include all available data from Group 5 up to this point.

Additional interim analyses may be performed during the study for the purpose of informing future vaccine-related decisions in a timely manner. The results will not influence the conduct of the study in terms of early termination or later safety or immunogenicity endpoint assessments.

The primary analysis will be performed on the main study only and will be done when all subjects in Groups 1 to 4 have completed the 21-day post-boost visit (Day 50 for Groups 1 and 3; Day 78 for Groups 2 and 4) or discontinued earlier. This analysis will include all available data from the main study up to this point.

The final analysis will be performed when all subjects from Groups 1 to 5 have completed the last study-related visit or discontinued earlier.

11.1. Analysis Sets

Main Study

The Full Analysis (FA) set includes all subjects who were randomized and received at least one dose of study vaccine, regardless of the occurrence of protocol deviations. Safety data will be analyzed based on the FA analysis set.

The Immunogenicity Response (IR) analysis set includes all randomized and vaccinated subjects, who have data from baseline and at least one post-vaccination immunogenicity blood draw.

The Per Protocol (PP) analysis set includes all randomized and vaccinated subjects, who received both the prime and boost vaccinations, have data from baseline and at least one post-vaccination immunogenicity blood draw, and have no major protocol violations.

Substudy

The Full Analysis (FA) set includes all subjects who received at least one dose of study vaccine, regardless of the occurrence of protocol deviations. Safety data will be analyzed based on the FA analysis set.

The Immunogenicity Response (IR) analysis set includes all vaccinated subjects, who have data from baseline and at least one post-vaccination immunogenicity blood draw.

The Per Protocol (PP) analysis set includes all vaccinated subjects, who received both the prime and boost vaccinations, have data from baseline and at least one post-vaccination immunogenicity blood draw, and have no major protocol violations.

11.2. Sample Size Determination

In each group (Groups 1 to 5), 15 subjects will receive active vaccine. Three subjects in each group in the main study (Groups 1 to 4) will receive placebo. The primary objective of this study is safety and tolerability.

The sample size is not based on formal hypothesis testing considerations, but is within the range of subjects as recommended by the International Conference on Harmonisation (ICH) for first-in-human products in this investigation. Placebo recipients are included in the main study for blinding purposes and safety analyses, and will provide control specimens for immunologic assays.

The sample size for this study will provide a preliminary safety and immunogenicity assessment. While mild to moderate vaccine reactions (local and systemic responses) are expected, adverse events that preclude further study vaccine administration or more serious events that would limit product development are not anticipated. With 15 subjects in the active vaccine groups, the observation of 0 such reactions would be associated with a one-sided 97.5% confidence upper limit that the true rate is less than 22%. Table 11 shows the probabilities of observing at least one adverse event at given true adverse event rates.

Table 11: Probability of Observing at Least One Adverse Event Given a True Adverse Event Incidence

	Probability of Observing at Least One Adverse Event
True Adverse Event Incidence	N=15
1%	14%
2.5%	32%
5%	54%
10%	79%
20%	96%

N: number of subjects receiving active vaccine

11.3. Subject Information

For all subjects, demographic characteristics (eg, age, height, weight, BMI, race, and sex) and screening/baseline characteristics (eg, physical examination, medical history) will be tabulated and summarized with descriptive statistics.

11.4. Immunogenicity Analyses

No formal hypothesis on immunogenicity will be tested. The analysis of immunogenicity will be done on the IR analysis set and on the PP analysis set in case more than 10% of the subjects from the IR analysis set are excluded from the PP analysis set. See Section 11.1, Analysis Sets for the definitions of the analysis sets.

Descriptive statistics (actual values, changes from references) will be calculated for continuous immunologic parameters at all time points. Graphical representations of changes in immunologic parameters will be prepared, as applicable. Frequency tabulations will be calculated for discrete (qualitative) immunologic parameters at all time points.

In addition, for the immunologic parameters the response patterns over time will be analyzed, taking into account within-subject correlations.

11.5. Safety Analyses

No formal statistical testing of safety data is planned. Safety data will be analyzed descriptively (including 95% confidence intervals [CIs], if applicable).

Baseline for all safety parameters will be defined as the last value before the prime vaccination.

Adverse Events (Including Reactogenicity)

The verbatim terms used in the CRF by investigators to report adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). All reported adverse events (solicited local, solicited systemic, and unsolicited) during the treatment period (ie, treatment-emergent adverse events, and adverse events that have worsened since baseline) will be included in the analysis. For each adverse event, the number and percentage of subjects who experience at least one occurrence of the given event will be summarized. Summaries, listings, datasets and/or subject narratives may be provided as appropriate, for those subjects who die, discontinue study vaccinations due to an adverse event, or experience a severe or serious adverse event. The analysis for solicited adverse events will be done on those subjects in the FA set for whom

reactogenicity assessments are available in the database. The analysis of unsolicited adverse events will be done based on the FA set.

Clinical Laboratory Tests

Laboratory data will be summarized by the type of laboratory test. Descriptive statistics (actual values and changes from reference) will be calculated for each laboratory analyte at baseline and at each scheduled time point. Graphical presentation of changes in laboratory tests will be made, as applicable. If the baseline value is not available, the value at screening will be used as baseline value. Laboratory abnormalities will be determined according to the toxicity grading tables included in Attachment 1, and in accordance with the normal ranges of the clinical laboratory. Laboratory abnormalities will be tabulated per treatment group and scheduled time point.

Vital Signs and Electrocardiograms

Descriptive statistics of heart rate, blood pressure (systolic and diastolic), oral body temperature and ECG parameters (QTcF and PR interval) values and changes from baseline will be summarized at each scheduled time point. The percentage of subjects with values beyond pre-specified limits will be summarized.

Physical Examination

Physical examination findings and changes from baseline will be summarized at each scheduled time point. Abnormalities will be listed. BMI will be calculated using the recording of height at screening. Body weight and BMI results will be tabulated and summarized descriptively.

11.6. Interim Analyses

Three interim analyses are planned in the main study for decision-making for future studies. The first interim analysis will assess safety data and will be performed when all subjects in the main study have completed the 7 days post-prime visit. The second interim analysis will be performed when all subjects in the Sentinel Cohorts and in Cohort 1 (26 subjects) have completed the 28 days post-prime visit to assess immunogenicity. The third interim analysis will be performed when all remaining subjects in Cohort 2 (46 subjects) have completed the 28 days post-prime visit to assess safety and immunogenicity. This analysis will include all available data from the main study up to this point.

Two interim analyses are planned in the substudy. The first interim analysis will assess safety data and will be performed when all subjects in Group 5 have completed the 7 days post-prime visit. The second interim analysis will be performed when all subjects have completed the 21 day post-boost visit (Day 36) or discontinued earlier. This analysis will include all available data from Group 5 up to this point.

Additional interim analyses may be performed during the study for the purpose of informing future vaccine-related decisions in a timely manner. The results will not influence the conduct of the study in terms of early termination or later safety or immunogenicity endpoint assessments.

11.7. Data Review Committee (DRC)

An internal DRC will be appointed by the sponsor before the start of the study and will operate according to its charter. The Principal Investigator will call a DRC meeting if any pausing rule is met at any time during the study.

The DRC will review all data that are deemed necessary to evaluate the adverse event(s), serious adverse event(s) and/or laboratory abnormality/ies that triggered the meeting and will reach a conclusion on further study conduct. The conclusions of the DRC will be communicated to the investigators, the IRB/IEC and the national regulatory authorities as appropriate. The sponsor agrees to abide by the decision of the DRC and any directives issued by the IRB/IEC or national regulatory authorities. In general, the DRC may unblind any amount of safety information needed to conduct their assessment.

The results of the interim analyses will also be communicated to the DRC.

The internal DRC will consist of at least one medical expert in the relevant therapeutic area and at least one statistician. DRC responsibilities, authorities, and procedures will be documented in its charter.

11.8. Pausing Rules

The Principal Investigator and the designated co-investigators will review the safety of enrolled subjects on an ongoing basis.

If any pausing rule is met, and following an internal safety review it is deemed appropriate to restart dosing, a request to restart dosing with pertinent data must be submitted to health authorities as a request for a substantial amendment. Communications from the DRC will be forwarded by the investigator to the IRB/IEC and by the sponsor to relevant health authorities.

The following pausing rules apply to the Sentinel Cohorts. These pausing rules will prevent the start of enrollment of Cohort 1 and will trigger a DRC meeting:

1. One subject experiences anaphylaxis or generalized urticaria clearly attributable to study vaccination within 24 hours of the prime vaccination; *OR*
2. One subject experiences a serious adverse event that is considered to be related to any of the study vaccines; *OR*
3. One subject experiences a severe (grade 3) unsolicited adverse event that is considered to be related to any of the study vaccines; *OR*
4. One subject experiences a severe (grade 3) solicited injection site reaction. (The size [measured in mm] of erythema will not be used as a pausing criterion); *OR*
5. One subject experiences a severe (grade 3) solicited systemic adverse event considered to be related to any of the study vaccines. (Subjective systemic adverse event corroborated by study personnel.); *OR*
6. Death of a subject.

The following pausing rules apply to Cohort 1 of any group in the main study. These pausing rules will prevent the start of enrollment of Cohort 2 and will trigger a DRC meeting:

1. One subject experiences anaphylaxis or generalized urticaria clearly attributable to study vaccination within 24 hours of prime vaccination; *OR*
2. One subject experiences a serious adverse event that is considered to be related to any of the study vaccines; *OR*
3. One subject experiences a severe (grade 3) unsolicited adverse event that is considered to be related to any of the study vaccines; *OR*
4. One subject experiences a severe (grade 3) solicited injection site reaction. (The size [measured in mm] of erythema will not be used as a pausing criterion); *OR*
5. One subject experiences a severe (grade 3) solicited systemic adverse event considered to be related to any of the study vaccines. (Subjective systemic adverse event corroborated by study personnel.); *OR*
6. Death of a subject.

Occurrence of any of the following events after initiation of Cohort 2 in any group in the main study will lead to suspension of further study vaccination in both the main and the substudy, and will trigger a meeting of the DRC to discuss study suspension or discontinuation of further vaccination:

1. One or more subjects experience a serious adverse event that is considered to be related to any of the study vaccines; *OR*
2. One or more subjects experience anaphylaxis or generalized urticaria clearly attributable to vaccination with study vaccine; *OR*
3. Two or more subjects experience a severe (grade 3) (non-serious) unsolicited adverse event that is considered to be related to any of the study vaccines; *OR*
4. Two or more subjects experience a severe (grade 3) (non-serious) laboratory abnormality (including unexplained hematuria) considered to be related to any of the study vaccines; *OR*
5. Two or more subjects who received at least one dose of study vaccine to date experience the same severe (grade 3) (non-serious) solicited injection site reaction. (The size [measured in mm] of erythema will not be used as a pausing criterion); *OR*
6. Two or more subjects who received at least one dose of study vaccine to date experience the same severe (grade 3) (non-serious) solicited systemic adverse event considered to be related to any of the study vaccines. (Subjective systemic adverse event corroborated by study personnel.); *OR*
7. Death of a subject.

For the events described above, the investigator notifies the sponsor's study responsible physician (or designee) immediately, and in all cases within 24 hours at the latest after the site observes, or

is notified of, the adverse events, and the study responsible physician (or contacted sponsor's representative) then notifies the DRC immediately. A thorough analysis of all grade 3 cases will be carried out by the investigator with the study responsible physician, irrespective of whether the criteria for pausing the study are met. If the case(s) is (are) deemed to meet the pausing rules, specified above, the DRC will convene within 3 business days to review these adverse events.

A subject will not be given the boost vaccination if he/she experiences:

1. Anaphylaxis clearly attributable to vaccination with study vaccine; *OR*
2. Generalized urticaria considered to be related to any of the study vaccines within 72 hours after administration of a study vaccine; *OR*
3. A serious adverse event that is considered to be related to any of the study vaccines; *OR*
4. A severe (grade 3) (non-serious) laboratory abnormality (including unexplained hematuria) that is considered to be related to any of the study vaccines; *OR*
5. A severe (grade 3) (non-serious) unsolicited adverse event that is considered to be related to any of the study vaccines; *OR*
6. A severe (grade 3) (non-serious) solicited injection site reaction that does not recover within 48 hours, *OR*
7. A severe (grade 3) (non-serious) solicited systemic adverse event considered to be related to any of the study vaccines that does not recover within 48 hours.

Vaccinations for an individual subject may be suspended for safety concerns other than those described above, at the discretion of the investigator if he/she feels the subject's safety may be threatened. The investigator may ask for a review meeting to be held for any single event or combination of multiple events which, in his/her professional opinion, jeopardize the safety of the subjects or the reliability of the data.

Vaccinations for the study may be suspended for safety concerns other than those described above or before pausing rules are met if in the judgment of investigator, subject safety may be threatened. The sponsor should be notified that the DRC will need to be convened.

If a pause is installed, vaccinations will be held until DRC review is complete. Resumption of vaccination may be determined by the DRC following cumulative review of the available safety data as outlined in the charter. The site will be allowed to resume activities upon receipt of a written notification from the sponsor. As applicable, the appropriate regulatory authorities will be informed in writing of the decision by the DRC to resume or discontinue study activities. The site is responsible for notifying their IRB/IEC according to local standards and regulations. The sponsor is responsible for notifying the regulatory authorities.

12. ADVERSE EVENT REPORTING

Timely, accurate, and complete reporting and analysis of safety information from clinical studies are crucial for the protection of subjects, investigators, and the sponsor, and are mandated by

regulatory agencies worldwide. The sponsor has established Standard Operating Procedures in conformity with regulatory requirements worldwide to ensure appropriate reporting of safety information; all clinical studies conducted by the sponsor or its affiliates will be conducted in accordance with those procedures.

12.1. Definitions

12.1.1. Adverse Event Definitions and Classifications

Adverse Event

An adverse event is any untoward medical occurrence in a clinical study subject administered a medicinal (investigational or non-investigational) product. An adverse event does not necessarily have a causal relationship with the treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non-investigational) product, whether or not related to that medicinal (investigational or non-investigational) product. (Definition per ICH)

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities.

Note: The sponsor collects adverse events starting with the signing of the ICF (see Section 12.3.1, All Adverse Events, for time of last adverse event recording).

Serious Adverse Event

A serious adverse event based on ICH and EU Guidelines on Pharmacovigilance for Medicinal Products for Human Use is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
  (The subject was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is a suspected transmission of any infectious agent via a medicinal product
- Is Medically Important*

* Medical and scientific judgment should be exercised in deciding whether expedited reporting is also appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should usually be considered serious.

If a serious and unexpected adverse event occurs for which there is evidence suggesting a causal relationship between the study vaccine and the event (eg, death from anaphylaxis), the event must be reported as a serious, unexpected suspected adverse reaction (SUSAR) (even after the study is over, if the sponsor, DRC or investigator becomes aware of them) by the sponsor to the Health Authorities and by the investigator to the IRB/IEC according to regulatory and local requirements.

Unlisted (Unexpected) Adverse Event/Reference Safety Information

An adverse event is considered unlisted if the nature or severity is not consistent with the applicable product reference safety information. For Ad26.ZEBOV and for MVA-BN-Filo, the expectedness of an adverse event will be determined by whether or not it is listed in the respective Investigator's Brochures and Addenda, if applicable.[12,13,14] For MVA-BN-Filo, for reporting purposes, all increases of troponin I will be considered unlisted.

Adverse Event Associated With the Use of the Study Vaccine

An adverse event is considered associated with the use of the study vaccine if the attribution is possibly, probably or very likely by the definitions listed in Section 12.1.2.

An adverse event is considered not associated with the use of the study vaccine if the attribution is not related or doubtful by the definitions listed in Section 12.1.2.

Adverse Events of Special Interest

Although not a single case of confirmed myocarditis and/or pericarditis has been reported to date following MVA-BN vaccination, due to particular concerns associated with traditional smallpox vaccines, the following adverse events of special interest are defined and must be reported to the appropriate sponsor contact person by study-site personnel within 24 hours of their knowledge of the event:

- Any cardiac sign or symptom developed since the prime vaccination
- ECG changes determined to be clinically significant

Any of the above will trigger investigation of troponin I and an increase of troponin I ≥2 x institutional upper limit of normal will be also considered adverse events of special interest.

Troponin I increase will always be considered unexpected for regulatory purposes in the current study.

12.1.2. Attribution Definitions

Every effort should be made by the investigator to explain any adverse event and to assess its potential causal relationship, ie, to administration of the study vaccine or to alternative causes (eg, natural history of an underlying diseases, concomitant therapies). This applies to all adverse events, whether serious or non-serious.

The investigator will use the following guidelines to assess the causal relationship of an adverse event to study vaccine:

Not Related

An adverse event that is not related to the use of study vaccine.

Doubtful

An adverse event for which an alternative explanation is more likely, eg, concomitant drug(s), concomitant disease(s), or the relationship in time suggests that a causal relationship is unlikely.

Possible

An adverse event that might be due to the use of study vaccine. An alternative explanation, eg, concomitant drug(s), concomitant disease(s), is inconclusive. The relationship in time is reasonable; therefore, the causal relationship cannot be excluded.

Probable

An adverse event that might be due to the use of study vaccine. The relationship in time is suggestive (eg, confirmed by dechallenge). An alternative explanation, eg, concomitant drug(s), concomitant disease(s), is less likely.

Very Likely

An adverse event that is listed as a possible adverse reaction and cannot be reasonably explained by an alternative explanation, eg, concomitant drug(s), concomitant disease(s). The relationship in time is very suggestive.

12.1.3. Severity Criteria

Adverse events and laboratory data will be coded for severity using the toxicity grading tables in Attachment 1. For adverse events not identified in the table, the following guidelines will apply:

Mild	Grade 1	Symptoms causing no or minimal interference with usual social and functional activities
Moderate	Grade 2	Symptoms causing greater than minimal interference with usual social and functional activities
Severe	Grade 3	Symptoms causing inability to perform usual social and functional activities

12.2. Special Reporting Situations

Safety events of interest on a sponsor study vaccine that may require expedited reporting and/or safety evaluation include, but are not limited to:

- Overdose of a sponsor study vaccine
- Inadvertent or accidental exposure to a sponsor study vaccine

- Medication error involving a sponsor product (with or without subject/patient exposure to the sponsor study vaccine, eg, name confusion)

Special reporting situations should be recorded in the CRF. Any special reporting situation that meets the criteria of a serious adverse event should be recorded on the serious adverse event page of the CRF.

12.3. Procedures

12.3.1. All Adverse Events

Adverse events and special reporting situations, whether serious or non-serious, will be collected from signing of the ICF onwards until 21 days post-boost (Day 36 for Group 5; Day 50 for Groups 1 and 3; Day 78 for Groups 2 and 4), and serious adverse events and adverse events related to blood draws will be collected from signing of the ICF onwards until the end of the study. Subjects will record symptoms of unsolicited and solicited local and systemic adverse events in the diary in the evening after each vaccination and then daily for the next 7 days.

Serious adverse events must be reported using the serious adverse event form. SUSARs will be reported even after the study is over, if the sponsor, the DRC or the investigator becomes aware of them. The sponsor will evaluate any safety information that is spontaneously reported by an investigator beyond the time frame specified in the protocol.

The investigator will monitor and analyze the study data including all adverse event and clinical laboratory data as they become available and will make determinations regarding the severity of the adverse experiences and their relation to study vaccine. All adverse events will be deemed related to study vaccine or not related to study vaccine, according to Section 12.1.2, Attribution Definitions. To ensure that all adverse events are captured in a timely manner, the CRFs will be entered in real-time and subjected to review to identify adverse events which may invoke pausing rules.

The investigator or designee must review both PIR and other adverse event CRFs to insure the prompt and complete identification of all events that require expedited reporting as serious adverse events, invoke pausing rules or are other serious and unexpected events.

All adverse events, regardless of seriousness, severity or presumed relationship to study vaccine, must be recorded using medical terminology in the source document and the CRF. Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology (eg, cough, runny nose, sneezing, sore throat, and head congestion should be reported as “upper respiratory infection”). The investigator must record in the CRF his/her opinion concerning the relationship of the adverse event to study vaccine. All measures required for the management of adverse events must be recorded in the source document and reported according to sponsor instructions.

The sponsor assumes responsibility for appropriate reporting of adverse events to the regulatory authorities. The sponsor will also report to the investigator (and the head of the investigational

institute where required) all serious adverse events that are unlisted (unexpected) and associated with the use of the study vaccine. The investigator (or sponsor where required) must report these events to the appropriate IEC/IRB that approved the protocol unless otherwise required and documented by the IEC/IRB.

Subjects will be provided with a "wallet (study) card" and instructed to carry this card with them for the duration of the study indicating the following:

- Study number
- Statement, in the local language(s), that the subject is participating in a clinical study
- Investigator's name and 24-hour contact telephone number
- Sponsor's name and 24-hour contact telephone number (for medical staff only)
- Site number
- Subject number
- Information about who should be contacted in case of emergency

12.3.2. Serious Adverse Events

All serious adverse events occurring during the study must be reported to the appropriate sponsor contact person by study-site personnel within 24 hours of their knowledge of the event.

Information regarding serious adverse events will be transmitted to the sponsor using the serious adverse event form, which must be completed and signed by a physician from the study site, and transmitted to the sponsor within 24 hours. The initial and follow-up reports of a serious adverse event should be made by facsimile (fax).

All serious adverse events that have not resolved by the end of the study, or that have not resolved upon discontinuation of the subject's participation in the study, must be followed until any of the following occurs:

- The event resolves
- The event stabilizes
- The event returns to baseline, if a baseline value/status is available
- The event can be attributed to agents other than the study vaccine or to factors unrelated to study conduct
- It becomes unlikely that any additional information can be obtained (subject or health care practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts)

Suspected transmission of an infectious agent by a medicinal product will be reported as a serious adverse event. Any event requiring hospitalization (or prolongation of hospitalization) that occurs during the course of a subject's participation in a study must be reported as a serious adverse event, except hospitalizations for the following:

- Hospitalizations not intended to treat an acute illness or adverse event (eg, social reasons such as pending placement in long-term care facility)
- Surgery or procedure planned before entry into the study (must be documented in the CRF). Note: Hospitalizations that were planned before the signing of the ICF, and where the underlying condition for which the hospitalization was planned has not worsened, will not be considered serious adverse events. Any adverse event that results in a prolongation of the originally planned hospitalization is to be reported as a new serious adverse event.

During the entire study, the cause of death of a subject, whether or not the event is expected or associated with the study vaccine, is considered a serious adverse event.

12.3.3. Pregnancy

All initial reports of pregnancy must be reported to the sponsor by the study-site personnel within 24 hours of their knowledge of the event using the appropriate pregnancy notification form. Abnormal pregnancy outcomes (eg, spontaneous abortion, stillbirth, and congenital anomaly) are considered serious adverse events and must be reported using the serious adverse event form. Any subject who becomes pregnant during the study must be promptly withdrawn from further study vaccination but should continue participation in the study for safety follow-up.

Because the effect of the study vaccine on sperm is unknown, pregnancies in partners of male subjects included in the study will be reported by the study-site personnel within 24 hours of their knowledge of the event using the appropriate pregnancy notification form.

Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required to be sent to the sponsor.

12.4. Contacting Sponsor Regarding Safety

The names (and corresponding telephone numbers) of the individuals who should be contacted regarding safety issues or questions regarding the study are listed on the Contact Information page(s), which will be provided as a separate document.

13. PRODUCT QUALITY COMPLAINT HANDLING

A product quality complaint (PQC) is defined as any suspicion of a product defect related to manufacturing, labeling, or packaging, ie, any dissatisfaction relative to the identity, quality, durability, or reliability of a product, including its labeling or package integrity. A PQC may have an impact on the safety and efficacy of the product. Timely, accurate, and complete reporting and analysis of PQC information from studies are crucial for the protection of subjects, investigators, and the sponsor, and are mandated by regulatory agencies worldwide. The sponsor has established procedures in conformity with regulatory requirements worldwide to ensure appropriate reporting of PQC information; all studies conducted by the sponsor or its affiliates will be conducted in accordance with those procedures.

13.1. Procedures

All initial PQCs must be reported to the sponsor by the study-site personnel within 24 hours after being made aware of the event.

If the defect is combined with a serious adverse event, the study-site personnel must report the PQC to the sponsor according to the serious adverse event reporting timelines (see Section 12.3.2, Serious Adverse Events). A sample of the suspected product should be maintained for further investigation if requested by the sponsor.

13.2. Contacting Sponsor Regarding Product Quality

The names (and corresponding telephone numbers) of the individuals who should be contacted regarding product quality issues are listed on the Contact Information page(s), which will be provided as a separate document.

14. STUDY VACCINE INFORMATION

14.1. Description of Study Vaccines

Ad26.ZEBOV

Ad26.ZEBOV is a monovalent recombinant, replication-incompetent Ad26-based vector that expresses the full length EBOV Mayinga GP and is produced in the human cell line PER.C6®.

The Ad26.ZEBOV vaccine will be supplied at a concentration of $1x10^{11}$ vp/mL in single-use vials as a frozen liquid to be thawed before use. Each stoppered and sealed glass vial contains an extractable volume of 0.5 mL. Refer to the Investigator's Brochure for a list of excipients.[12]

The Ad26.ZEBOV vaccine is manufactured by Crucell Holland BV, The Netherlands.

MVA-BN-Filo

MVA-BN-Filo is a recombinant multivalent vaccine intended for active immunization against Ebola and Marburg virus infection. MVA-BN-Filo is strongly attenuated; the vaccine is propagated in primary chicken embryo fibroblast (CEF) cells and does not replicate in human cells.

The MVA-BN-Filo vaccine is supplied at a concentration of $8.8x10^{8}$ $TCID_{50}$/mL (release titer) in 2-mL glass vials as a frozen liquid suspension to be thawed before use. Each vial contains an extractable volume of 0.5 mL. Refer to the Investigator's Brochure for a list of excipients.[14]

A diluent consisting of tris buffered saline (10 mM Tris, 140 mM NaCl, pH 7.7) will be supplied.

The MVA-BN-Filo vaccine is manufactured by Bavarian Nordic A/S, Denmark.

Placebo

The placebo supplied for this study will be formulated as a sterile 0.9% saline for injection (as commercially available).

14.2. Packaging and Labeling

All study vaccines will be manufactured and packaged in accordance with Good Manufacturing Practice (GMP). All study vaccines will be packaged and labeled under the responsibility of the sponsor. No study vaccine can be repacked or relabeled without prior approval from the sponsor.

Further details for study vaccine packaging and labeling can be found in the Site Investigational Product Procedures Manual.

14.3. Preparation, Handling and Storage

Study vaccine must be stored at controlled temperatures: Ad26.ZEBOV vials must be stored at ≤ –65°C and MVA-BN-Filo vials must be stored at –80°C ± 10°C.

Vials must be stored in a secured location with no access for unauthorized personnel. The study freezer must be equipped with a continuous temperature monitor and alarm. Study freezers should be equipped with back-up power systems. In the event that study vaccine is exposed to temperatures outside the specified temperature ranges, all relevant data will be sent to the sponsor to determine if the affected study vaccine can be used or will be replaced. The affected study vaccine must be quarantined and not used until further instruction from the sponsor is received.

An unblinded pharmacist/qualified staff member will prepare all doses for vaccine administration and provide it to the clinic for dispensing in a blinded way (main study only) by an independent study vaccine administrator (see Definitions of Terms). All other site members and subjects in the main study, will remain blinded to the study vaccine administered. To preserve blinding, the pharmacist/qualified staff member will place a blinding tape on the syringe to mask its content.

Ad26.ZEBOV Preparation

Details on the preparation, the holding time and storage conditions from the time of preparation to delivery of Ad26.ZEBOV are provided in the Site Investigational Product Procedures Manual.

MVA-BN-Filo Preparation

Details on the preparation, the holding time and storage conditions from the time of preparation to delivery of MVA-BN-Filo are provided in the Site Investigational Product Procedures Manual.

14.4. Study Vaccine Accountability

The investigator is responsible for ensuring that all study vaccine received at the site is inventoried and accounted for throughout the study. The study vaccine administered to the subject must be documented on the study vaccine accountability form. All study vaccine will be stored and disposed of according to the sponsor's instructions. Study-site personnel must not combine contents of the study vaccine containers.

Study vaccine must be handled in strict accordance with the protocol and the container label, and must be stored at the study site in a limited-access area or in a locked cabinet under appropriate environmental conditions. Unused study vaccine must be available for verification by the

sponsor's study site monitor during on-site monitoring visits. The return to the sponsor of unused study vaccine will be documented on the study vaccine return form. When the study site is an authorized destruction unit and study vaccine supplies are destroyed on-site, this must also be documented on the study vaccine return form.

Potentially hazardous materials, such as used ampules, needles, syringes and vials containing hazardous liquids, should be disposed of immediately in a safe manner and therefore will not be retained for study vaccine accountability purposes.

Study vaccine should be dispensed under the supervision of the investigator or qualified member of the study-site personnel, or by the pharmacist/qualified staff member. Study vaccine will be supplied only to subjects participating in the study. Returned study vaccine must not be dispensed again, even to the same subject. Study vaccine may not be relabeled or reassigned for use by other subjects. The investigator agrees neither to dispense the study vaccine from, nor store it at, any site other than the study sites agreed upon with the sponsor.

15. STUDY-SPECIFIC MATERIALS

The investigator will be provided with the following supplies:

- Investigator Brochures and Addendum for Ad26.ZEBOV and MVA-BN-Filo
- Site Investigational Product Procedures Manual
- Laboratory Manual
- IWRS Manual
- Electronic Data Capture (eDC) Manual/electronic CRF Completion Guidelines and Randomization Instructions
- Sample ICF
- Subject diaries
- Rulers, thermometers
- Subject wallet cards
- Recruitment tools, as applicable

16. ETHICAL ASPECTS

16.1. Study-Specific Design Considerations

Potential subjects will be fully informed of the risks and requirements of the study and, during the study, subjects will be given any new information that may affect their decision to continue participation. They will be told that their consent to participate in the study is voluntary and may be withdrawn at any time with no reason given and without penalty or loss of benefits to which they would otherwise be entitled. Only subjects who are fully able to understand the risks, benefits, and potential adverse events of the study, and provide their consent voluntarily will be enrolled.

The total blood volume to be collected is expected to be approximately 505 mL (Groups 1 and 3) or 565 mL (Groups 2 and 4) from each subject receiving placebo, and 685 mL (Groups 1 and 3) or 745 mL (Group 2 and 4) from each subject receiving active vaccine. The total blood volume to be collected in Group 5 is expected to be approximately 685 mL.

16.2. Regulatory Ethics Compliance

16.2.1. Investigator Responsibilities

The investigator is responsible for ensuring that the study is performed in accordance with the protocol, current ICH guidelines on Good Clinical Practice (GCP), and applicable regulatory and country-specific requirements.

Good Clinical Practice is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and well-being of study subjects are protected, consistent with the principles that originated in the Declaration of Helsinki, and that the study data are credible.

16.2.2. Independent Ethics Committee or Institutional Review Board

Before the start of the study, the investigator (or sponsor where required) will provide the IEC/IRB with current and complete copies of the following documents (as required by local regulations):

- Final protocol and, if applicable, amendments
- Sponsor-approved ICF (and any other written materials to be provided to the subjects)
- Investigator's Brochure (or equivalent information) and amendments/addenda
- Sponsor-approved subject recruiting materials
- Information on compensation for study-related injuries or payment to subjects for participation in the study, if applicable
- Investigator's curriculum vitae or equivalent information (unless not required, as documented by the IEC/IRB)
- Information regarding funding, name of the sponsor, institutional affiliations, other potential conflicts of interest, and incentives for subjects
- Any other documents that the IEC/IRB requests to fulfill its obligation

This study will be undertaken only after the IEC/IRB has given full approval of the final protocol, amendments (if any, excluding the ones that are purely administrative, with no consequences for subjects, data or study conduct), the ICF, applicable recruiting materials, and subject compensation programs, and the sponsor has received a copy of this approval. This approval letter must be dated and must clearly identify the IEC/IRB and the documents being approved.

During the study the investigator (or sponsor where required) will send the following documents and updates to the IEC/IRB for their review and approval, where appropriate:

- Protocol amendments (excluding the ones that are purely administrative, with no consequences for subjects, data or study conduct)
- Revision(s) to ICF and any other written materials to be provided to subjects
- If applicable, new or revised subject recruiting materials approved by the sponsor
- Revisions to compensation for study-related injuries or payment to subjects for participation in the study, if applicable
- New edition(s) of the Investigator's Brochure and amendments/addenda
- Summaries of the status of the study at intervals stipulated in guidelines of the IEC/IRB (at least annually)
- Reports of adverse events that are serious, unlisted/unexpected, and associated with study vaccine
- New information that may adversely affect the safety of the subjects or the conduct of study vaccine
- Deviations from or changes to the protocol to eliminate immediate hazards to the subjects
- Report of deaths of subjects under the investigator's care
- Notification if a new investigator is responsible for the study at the site
- Development Safety Update Report and Line Listings, where applicable
- Any other requirements of the IEC/IRB

For all protocol amendments (excluding the ones that are purely administrative, with no consequences for subjects, data or study conduct), the amendment and applicable ICF revisions must be submitted promptly to the IEC/IRB for review and approval before implementation of the change(s).

At least once a year, the IEC/IRB will be asked to review and reapprove this study. The re-approval should be documented in writing (excluding the ones that are purely administrative, with no consequences for subjects, data, or study conduct).

At the end of the study, the investigator (or sponsor where required) will notify the IEC/IRB about the study completion.

16.2.3. Informed Consent

Each subject must give written consent according to local requirements after the nature of the study has been fully explained. The ICF must be signed before performance of any study-related activity. The ICF that is/are used must be approved by both the sponsor and by the reviewing IEC/IRB and be in a language that the subject can read and understand. The informed consent should be in accordance with principles that originated in the Declaration of Helsinki, current ICH and GCP guidelines, applicable regulatory requirements, and sponsor policy.

Before enrollment into the study, the investigator or an authorized member of the study-site personnel must explain to potential subjects the aims, methods, reasonably anticipated benefits, and potential hazards of the study, and any discomfort participation in the study may entail. Subjects will be informed that their participation is voluntary and that they may withdraw consent to participate at any time. They will be informed that choosing not to participate will not affect the care the subject will receive. Finally, they will be told that the investigator will maintain a subject identification register for the purposes of long-term follow up if needed and that their records may be accessed by health authorities and authorized sponsor personnel without violating the confidentiality of the subject, to the extent permitted by the applicable law(s) or regulations. By signing the ICF the subject is authorizing such access and agrees to allow his or her study physician to recontact the subject for the purpose of obtaining consent for additional safety evaluations, if needed.

The subject will be given sufficient time to read the ICF and the opportunity to ask questions. After this explanation and before entry into the study, consent should be appropriately recorded by means of the subject's personally dated signature. After having obtained the consent, a copy of the ICF must be given to the subject.

Subjects will be asked for consent to provide optional samples for research (where local regulations permit). After informed consent for the study is appropriately obtained, subjects will be asked to sign and personally date a separate ICF indicating agreement to participate in the optional research component. Refusal to participate in the optional research will not result in ineligibility for the study. A copy of this signed ICF will be given to the subject.

16.2.4. Privacy of Personal Data

The collection and processing of personal data from subjects enrolled in this study will be limited to those data that are necessary to fulfill the objectives of the study.

These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data agree to keep the identity of subjects confidential.

The informed consent obtained from the subject includes explicit consent for the processing of personal data and for the investigator/institution to allow direct access to his or her original medical records (source data/documents) for study-related monitoring, audit, IEC/IRB review, and regulatory inspection. This consent also addresses the transfer of the data to other entities and to other countries.

The subject has the right to request through the investigator access to his or her personal data and the right to request rectification of any data that are not correct or complete. Reasonable steps will be taken to respond to such a request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations.

Exploratory research is not conducted under standards appropriate for the return of data to subjects. In addition, the sponsor cannot make decisions as to the significance of any findings resulting from exploratory research. Therefore, exploratory research data will not be returned to subjects or investigators, unless required by law or local regulations. Privacy and confidentiality of data generated in the future on stored samples will be protected by the same standards applicable to all other clinical data.

16.2.5. Long-Term Retention of Samples for Additional Future Research

Each study subject will be asked to consent voluntarily for their blood samples to be stored for other research studies that may be done after this study is completed. Future testing may involve DNA/RNA tests. Subjects unwilling to have their blood samples stored for future use, can consent to participate in this study only without having their blood samples stored for future testing (see also Section 10.4, Withdrawal From the Study). In such case, their blood samples will be destroyed after all the tests specified for this study have been concluded.

All samples, for which consent has been obtained and for which additional material is available after study-specified testing is complete, will be stored for future testing. Applicable approvals will be sought before any such samples are used for analysis not specified in the protocol or a protocol amendment approved by the IEC/IRB.

Stored samples will be coded throughout the sample storage and analysis process and will not be labeled with personal identifiers.

16.2.6. Country Selection

This study will only be conducted in those countries where the intent is to launch or otherwise help ensure access to the developed product, unless explicitly addressed as a specific ethical consideration in Section 16.1, Study-Specific Design Considerations.

17. ADMINISTRATIVE REQUIREMENTS

17.1. Protocol Amendments

Neither the investigator nor the sponsor will modify this protocol without a formal amendment by the sponsor. All protocol amendments must be issued by the sponsor, and signed and dated by the investigator. Protocol amendments must not be implemented without prior IEC/IRB approval, or when the relevant competent authority has raised any grounds for non-acceptance, except when necessary to eliminate immediate hazards to the subjects, in which case the amendment must be promptly submitted to the IEC/IRB and relevant competent authority. Documentation of amendment approval by the investigator and IEC/IRB must be provided to the sponsor. When the change(s) involves only logistic or administrative aspects of the study, the IRB (and IEC where required) only needs to be notified.

During the course of the study, in situations where a departure from the protocol is unavoidable, the investigator or other physician in attendance will contact the appropriate sponsor representative (see Contact Information page(s) provided separately). Except in emergency

situations, this contact should be made before implementing any departure from the protocol. In all cases, contact with the sponsor must be made as soon as possible to discuss the situation and agree on an appropriate course of action. The data recorded in the CRF and source documents will reflect any departure from the protocol, and the source documents will describe this departure and the circumstances requiring it.

17.2. Regulatory Documentation

17.2.1. Regulatory Approval/Notification

This protocol and any amendment(s) must be submitted to the appropriate regulatory authorities in each respective country, if applicable. A study may not be initiated until all local regulatory requirements are met.

17.2.2. Required Prestudy Documentation

The following documents must be provided to the sponsor before shipment of study vaccine to the study site:

- Protocol and amendment(s), if any, signed and dated by the principal investigator
- A copy of the dated and signed (or sealed, where appropriate per local regulations), written IEC/IRB approval of the protocol, amendments, ICF, any recruiting materials, and if applicable, subject compensation programs. This approval must clearly identify the specific protocol by title and number and must be signed (or sealed, where appropriate per local regulations) by the chairman or authorized designee.
- Name and address of the IEC/IRB, including a current list of the IEC/IRB members and their function, with a statement that it is organized and operates according to GCP and the applicable laws and regulations. If accompanied by a letter of explanation, or equivalent, from the IEC/IRB, a general statement may be substituted for this list. If an investigator or a member of the study-site personnel is a member of the IEC/IRB, documentation must be obtained to state that this person did not participate in the deliberations or in the vote/opinion of the study.
- Regulatory authority approval or notification, if applicable
- Signed and dated statement of investigator (eg, Form Food and Drug Administration [FDA] 1572), if applicable
- Documentation of investigator qualifications (eg, curriculum vitae)
- Completed investigator financial disclosure form from the principal investigator, where required
- Signed and dated clinical trial agreement, which includes the financial agreement
- Any other documentation required by local regulations

The following documents must be provided to the sponsor before enrollment of the first subject:

- Completed investigator financial disclosure forms from all sub-investigators, where required

- Documentation of sub-investigator qualifications (eg, curriculum vitae)
- Name and address of any local laboratory conducting tests for the study, and a dated copy of current laboratory normal ranges for these tests, if applicable
- Local laboratory documentation demonstrating competence and test reliability (eg, accreditation/license), if applicable

17.3. Subject Identification, Enrollment, and Screening Logs

The investigator agrees to complete a subject identification and enrollment log to permit easy identification of each subject during and after the study. This document will be reviewed by the sponsor study-site contact for completeness.

The subject identification and enrollment log will be treated as confidential and will be filed by the investigator in the study file. To ensure subject confidentiality, no copy will be made. All reports and communications relating to the study will identify subjects by subject identification and date of birth. In cases where the subject is not randomized into the study, the date seen and date of birth will be used.

The investigator must also complete a subject screening log, which reports on all subjects who were seen to determine eligibility for inclusion in the study.

17.4. Source Documentation

At a minimum, source documentation must be available for the following to confirm data collected in the CRF: subject identification, eligibility, and study identification; study discussion and date of signed informed consent; dates of visits; results of safety and efficacy parameters as required by the protocol; record of all adverse events and follow-up of adverse events; concomitant medication; study vaccine receipt/dispensing/return records; study vaccine administration information; and date of study completion and reason for early discontinuation of study vaccine or withdrawal from the study, if applicable.

In addition, the author of an entry in the source documents should be identifiable.

At a minimum, the type and level of detail of source data available for a subject should be consistent with that commonly recorded at the study site as a basis for standard medical care. Specific details required as source data for the study will be reviewed with the investigator before the study and will be described in the monitoring guidelines (or other equivalent document).

The subject diary will be considered a source document. Information from the diary provided to subjects to record symptoms of unsolicited and solicited local and systemic adverse events until 7 days after each vaccination, will be reviewed by the investigator or designee to transcribe into the relevant parts of the CRF as described in the CRF Completion Guidelines.

17.5. Case Report Form Completion

CRFs are provided for each subject in electronic format.

eDC will be used for this study. The study data will be transcribed by study-site personnel from the source documents onto an electronic CRF, and transmitted in a secure manner to the sponsor within the timeframe agreed upon between the sponsor and the study site. The electronic file will be considered to be the CRF.

All data relating to the study must be recorded in CRFs prepared by the sponsor. Data must be entered into CRFs in English. Study site personnel must complete the CRF as soon as possible after a subject visit, and the forms should be available for review at the next scheduled monitoring visit.

The investigator must verify that all data entries in the CRFs are accurate and correct.

All CRF entries, corrections, and alterations must be made by the investigator or other authorized study-site personnel. If necessary, queries will be generated in the eDC tool. The investigator or study-site personnel must adjust the CRF (if applicable) and complete the query.

If corrections to a CRF are needed after the initial entry into the CRF, this can be done in 3 different ways:

- Study site personnel can make corrections in the eDC tool at their own initiative or as a response to an auto query (generated by the eDC tool).
- Study site manager can generate a query for resolution by the study-site personnel.
- Clinical data manager can generate a query for resolution by the study-site personnel.

17.6. Data Quality Assurance/Quality Control

Steps to be taken to ensure the accuracy and reliability of data include the selection of qualified investigators and appropriate study sites, review of protocol procedures with the investigator and study-site personnel before the study, and periodic monitoring visits by the sponsor. Written instructions will be provided for collection, handling, storage, and shipment of samples.

Guidelines for CRF completion will be provided and reviewed with study-site personnel before the start of the study. The sponsor will review CRFs for accuracy and completeness during on-site monitoring visits and after transmission to the sponsor; any discrepancies will be resolved with the investigator or designee, as appropriate. After upload of the data into the study database they will be verified for accuracy and consistency with the data sources.

17.7. Record Retention

In compliance with the ICH/GCP guidelines, the investigator/institution will maintain all CRFs and all source documents that support the data collected from each subject, as well as all study documents as specified in ICH/GCP Section 8, Essential Documents for the Conduct of a Clinical Trial, and all study documents as specified by the applicable regulatory requirement(s). The investigator/institution will take measures to prevent accidental or premature destruction of these documents.

Essential documents must be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents will be retained for a longer period if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

If the responsible investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. The sponsor must be notified in writing of the name and address of the new custodian. Under no circumstance shall the investigator relocate or dispose of any study documents before having obtained written approval from the sponsor.

If it becomes necessary for the sponsor or the appropriate regulatory authority to review any documentation relating to this study, the investigator/institution must permit access to such reports.

17.8. Monitoring

The sponsor will perform on-site monitoring visits as frequently as necessary. The monitor will record dates of the visits in a study site visit log that will be kept at the study site. The first post-initiation visit will be made as soon as possible after enrollment has begun. At these visits, the monitor will compare the data entered into the CRFs with the hospital or clinic records (source documents). The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the CRF are known to the sponsor and study-site personnel and are accessible for verification by the sponsor study-site contact. If electronic records are maintained at the study site, the method of verification must be discussed with the study-site personnel.

Direct access to the source documentation (medical records) must be allowed for the purpose of verifying that the data recorded in the CRF are consistent with the original source data. Findings from this review of CRFs and source documents will be discussed with the study-site personnel. The sponsor expects that, during the monitoring visits, the relevant study-site personnel will be available, source documentation will be accessible, and a suitable environment will be provided for review of study-related documents. The monitor will meet with the investigator on a regular basis during the study to provide feedback on the study conduct.

There will be independent monitoring of the pharmacy and preparation of study vaccines by an unblinded monitor (independent drug monitor); regular monitors will be blinded.

In addition to on-site monitoring visits, remote contacts can occur. It is expected that during these remote contacts, study-site personnel will be available to provide an update on the progress of the study at the site.

17.9. Study Completion/Termination

17.9.1. Study Completion

The study is considered completed with the last visit for the last subject participating in the study. The final data from the study site will be sent to the sponsor (or designee) after completion of the final subject visit at that study site, in the time frame specified in the Clinical Trial Agreement.

17.9.2. Study Termination

The sponsor reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IEC/IRB or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of subjects by the investigator
- Discontinuation of further study vaccine development

17.10. On-Site Audits

Representatives of the sponsor's clinical quality assurance department may visit the study site at any time during or after completion of the study to conduct an audit of the study in compliance with regulatory guidelines and company policy. These audits will require access to all study records, including source documents, for inspection and comparison with the CRFs. Subject privacy must, however, be respected. The investigator and study-site personnel are responsible for being present and available for consultation during routinely scheduled study-site audit visits conducted by the sponsor or its designees.

Similar auditing procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The investigator should immediately notify the sponsor if he or she has been contacted by a regulatory agency concerning an upcoming inspection.

17.11. Use of Information and Publication

All information, including but not limited to information regarding the combination regimen of Ad26.ZEBOV and MVA-BN-Filo or the sponsor's operations (eg, patent application, formulas, manufacturing processes, basic scientific data, prior clinical data, formulation information) supplied by the sponsor to the investigator and not previously published, and any data, including

exploratory research data, generated as a result of this study, are considered confidential and remain the sole property of the sponsor. The investigator agrees to maintain this information in confidence and use this information only to accomplish this study, and will not use it for other purposes without the sponsor's prior written consent.

The investigator understands that the information developed in the study will be used by the sponsor in connection with the continued development of the combination regimen of Ad26.ZEBOV and MVA-BN-Filo, and thus may be disclosed as required to other clinical investigators or regulatory agencies. To permit the information derived from the clinical studies to be used, the investigator is obligated to provide the sponsor with all data obtained in the study.

The results of the study will be reported in a Clinical Study Report generated by the sponsor and will contain CRF data from all study sites that participated in the study. Recruitment performance or specific expertise related to the nature and the key assessment parameters of the study will be used to determine a coordinating investigator. Results of exploratory analyses performed after the Clinical Study Report has been issued will be reported in a separate report and will not require a revision of the Clinical Study Report. Study subject identifiers will not be used in publication of results. Any work created in connection with performance of the study and contained in the data that can benefit from copyright protection (except any publication by the investigator as provided for below) shall be the property of the sponsor as author and owner of copyright in such work.

Arrangements on publication policy will be addressed in the Clinical Trial Agreement.

Registration of Clinical Studies and Disclosure of Results

The sponsor will register and/or disclose the existence of and the results of clinical studies as required by law.

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Attachment 1: DMID Toxicity Table for Use in Trials Enrolling Healthy Adults (2014)

The abbreviations used in the following tables are:

ALT: alanine aminotransferase; aPTT: activated partial thromboplastin time; AST: aspartate aminotransferase; AV block: atrioventricular block; bpm: beats per minute; BUN: blood urea nitrogen; CK: creatine kinase; CPK: creatine phosphokinase; FEV_1: forced expiratory volume in 1 second; g: gram; HI: high; HPF: high power field; IU: international unit; IV: intravenous; K/CUMM: $x10^3/mm^3$; LLN: lower limit of normal; LO: low; mEq: milliequivalent; mm Hg: millimeter of mercury; ms: millisecond; N: normal; PT: prothrombin time; PTT: partial thromboplastin time; QTc: QT-interval corrected for heart rate; QTcB: Bazett's corrected QT interval; QTcF: Fridericia's corrected QT interval; RBC: red blood cell; Rx: therapy; s: second; U: unit; ULN: upper limit of normal

CLINICAL ADVERSE EVENTS

Cardiovascular	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Arrhythmia		Asymptomatic, transient signs, no Rx required	Recurrent/persistent; symptomatic Rx required
Hemorrhage, blood loss	Estimated blood loss ≤100 mL	Estimated blood loss >100 mL, no transfusion required	Transfusion required
QTcF (Fridericia's correction) [a] or QTcB (Bazett's correction)	Asymptomatic, QTc interval 450-479 ms, *OR* Increase in interval<30 ms above baseline	Asymptomatic, QTc interval 480-499 ms, *OR* Increase in interval 30-50 ms above baseline	Asymptomatic, QTc interval ≥500 ms, *OR* Increase in interval ≥60 ms above baseline
PR interval (prolonged)	PR interval 0.21-0.25 s	PR interval >0.25 s	Type II 2nd degree AV block *OR* Ventricular pause >3.0 s
Respiratory	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Cough	Transient-no treatment	Persistent cough	Interferes with daily activities
Bronchospasm, acute	Transient; no treatment; FEV_1 71%-80% of peak flow	Requires treatment; normalizes with bronchodilator; FEV_1 60%-70% (of peak flow)	No normalization with bronchodilator; FEV_1 <60% of peak flow
Dyspnea	Does not interfere with usual and social activities	Interferes with usual and social activities, no treatment	Prevents daily and usual social activity or requires treatment

[a] Inclusion dependent upon protocol requirements.

Gastrointestinal	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Nausea	No interference with activity	Some interference with activity	Prevents daily activities
Vomiting	No interference with activity or 1-2 episodes/24 hours	Some interference with activity or >2 episodes/24 hours	Prevents daily activity or requires IV hydration
Diarrhea	2-3 loose or watery stools or <400 g/24 hours	4-5 loose or watery stools or 400-800 g/24 hours	6 or more loose or watery stools or >800 g/24 hours or requires IV hydration
Reactogenicity	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Local reactions			
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever >24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity
Tenderness	Discomfort only to touch	Discomfort with movement	Significant discomfort at rest
Erythema/redness [a]	2.5-5 cm	5.1-10 cm	>10 cm
Induration/swelling [b]	2.5-5 cm and does not interfere with activity	5.1-10 cm or interferes with activity	>10 cm or prevents daily activity
Systemic reactions			
Allergic reaction	Pruritus without rash	Localized urticaria	Generalized urticaria; angioedema or anaphylaxis
Headache	No interference with activity	Repeated use of non-narcotic pain reliever >24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity
All other conditions	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention

[a] In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

[b] Induration/swelling should be evaluated and graded using the functional scale as well as the actual measurement.

LABORATORY AND VITAL SIGNS TOXICITY GRADING

Blood, Serum, or Plasma Chemistries [a,b]	LO/HI/N [c]	Mild (Grade 1) [d]	Moderate (Grade 2)	Severe (Grade 3)
Sodium (mEq/L or mmol/L)	LO	132-<LLN	130-131	<130
	HI	>ULN-148	149-150	>150
Potassium (mEq/L or mmol/L)	LO	>ULN-3.1	<3.1-3.0	<3.0
	HI	>ULN-5.2	5.3-5.4	>5.4
Glucose (mg/dL)	LO	65-67	55-64	<55
	HI [e]	>ULN-120	121-130	>130
	HI [f]	140-159	160-200	>200
Blood urea nitrogen	HI	23-26 (mg/dL) or 8.3-9.4 (mmol/L)	27-31 (mg/dL) or 9.5- 11.2 (mmol/L)	>31 (mg/dL) or >11.2 (mmol/L)
Creatinine	N	>ULN-1.7 (mg/dL) or >ULN-151 (µmol/L)	1.8-2.0 (mg/dL) or 152-177 (µmol/L)	>2.0 (mg/dL) or > 177 (µmol/L)
Calcium (mg/dL)	LO	8.0-<LLN	7.5-7.9	<7.5
	HI	>ULN-11.0	11.1-11.5	>11.5
Magnesium (mg/dL)	LO	1.3-1.5	1.1-1.2	<1.1
Phosphorous (mg/dL)	LO	2.3-2.5	2.0-2.2	<2.0
Creatinine kinase (CPK or CK) (IU/L)	N	400-1000	1001-1500	>1500
Albumin (g/dL)	LO	2.8-3.0	2.5-2.7	<2.5
Total protein (g/dL)	LO	5.2-<LLN	5.0-5.4	<5.0
Alkaline phosphatase (U/L)	N	132-240	241-360	>360
AST (U/L)	HI	44-105	106-175	>175
ALT (U/L)	HI	44-105	106 -175	>175
Bilirubin, serum total (mg/dL)	HI	1.3-2.0	2.1-2.5	>2.5
Bilirubin, serum total (mg/dL) when ALT ≥105 (Hy's law)	HI	1.3-1.5	1.6-2.0	>2.0
Amylase (U/L)	N	200-270	271-360	>360
Lipase (U/L)	N	176-270	271-360	>360

[a] Depending upon the laboratory used, references ranges, eligibility ranges and grading may be split out by sex and/or age.

[b] Cardiac troponin I increase by factor: >ULN-<2.0xULN; ≥2.0-<5.0xULN; ≥5.0xULN. (This footnote is added by the sponsor).

[c] Low, High, Not Graded.

[d] If initial bound of grade 1 has gap from reference range or eligibility range, calculations based on the New England Journal of Medicine (NEJM) reference ranges.

[e] Fasting.

[f] Non-fasting.

Hematology	LO/HI/N [a]	Mild (Grade 1) [b]	Moderate (Grade 2)	Severe (Grade 3)
Hemoglobin (women) (g/dL)	LO	11.0-11.5	9.5-10.9	<9.5
Hemoglobin (men) (g/dL)	LO	12.0-12.5	10.0-11.9	<10.0
White blood cell count (K/CUMM)	HI	11.00-15.00	15.00-20.00	>20.00
	LO	2.50-3.50	1.50-2.49	<1.50
Lymphocytes (K/CUMM)	LO	0.75-1.00	0.50-0.75	<0.5
Neutrophils (K/CUMM)	LO	1.50-2.00	1.00-1.49	<1.00
Eosinophils (K/CUMM)	HI	0.50-0.75	0.75-1.50	>1.50
Platelets (K/CUMM)	LO	120-130	100-120	<100
Coagulation				
Prothrombin time (PT, seconds)	HI	> ULN-14.4	14.5-15.7	>15.7
Partial thromboplastin time (PTT or aPTT, seconds)	HI	>ULN-42.1	42.2-50.0	>50.0
Fibrinogen (mg/dL)	HI	>ULN-500	501-600	>600
	LO	<LLN-140	125-139	<125
Urine*				
Protein (dipstick)	HI	1+	2+	>2+
Glucose (dipstick)	HI	1+	2+	>2+
Blood (microscopic) - red blood cells per high power field (RBC/HPF)	HI	5-10	11-50	>50 and/or gross blood

[a] Low, High, Not Graded.

[b] If initial bound of grade 1 has gap from reference range or eligibility range, calculations based on the New England Journal of Medicine (NEJM) reference ranges.

Vital Signs	LO/HI/N [a]	Mild (Grade 1) [b]	Moderate (Grade 2)	Severe (Grade 3)
Fever (°C)[c]	HI	38.0-38.4	38.5-38.9	>38.9
Fever (°F)	HI	100.4-101.1	101.2-102.0	>102.1
Tachycardia - beats per minute	HI	101-115	116-130	>130 or ventricular dysrhythmias
Bradycardia - beats per minute	LO	50-54 or 45-50 bpm if baseline <60 bpm	45-49 or 40-44 if baseline <60 bpm	<45 or <40 bpm if baseline <60 bpm
Hypertension (systolic) - mm Hg[d]	HI	141-150	151-160	>160
Hypertension (diastolic) - mm Hg	HI	91-95	96-100	>100
Hypotension (systolic) - mm Hg	LO	85-89	80-84	<80
Tachypnea - breaths per minute	HI	23-25	26-30	>30

[a] Low, High, Not Graded.

[b] If initial bound of grade 1 has gap from reference range or eligibility range, calculations based on the New England Journal of Medicine (NEJM) reference ranges.

[c] Oral temperature; no recent hot or cold beverages or smoking. A protocol should select either °C or °F for inclusion.

[d] Assuming subject is awake, resting, and supine; for adverse events, 3 measurements on the same arm with concordant results.

INVESTIGATOR AGREEMENT

I have read this document and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein and will complete the study within the time designated.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study vaccine, the conduct of the study, and the obligations of confidentiality.

Coordinating Investigator (where required):

Name (typed or printed): _____

Institution and Address: _____

_____

_____

_____

Signature: _____ Date: _____

(Day Month Year)

Principal (Site) Investigator:

Name (typed or printed): _____

Institution and Address: _____

_____

_____

_____

Telephone Number: _____

Signature: _____ Date: _____

(Day Month Year)

Sponsor's Responsible Medical Officer:

Name (typed or printed): Macaya Douoguih, MD

Institution: Crucell Holland BV

Signature: [electronic signature appended at the end of the protocol] Date: _____

(Day Month Year)

Note: If the address or telephone number of the investigator changes during the course of the study, written notification will be provided by the investigator to the sponsor, and a protocol amendment will not be required.

LAST PAGE

SIGNATURES

Signed by	Date	Justification
Macaya Douoguih	03Mar2015, 19:50:29 PM, UTC	Document Approval