A Systems Biology Phase 1 Evaluation of the Safety, Reactogenicity, and Immunogenicity of Chimpanzee Adenovirus Type 3- vectored *Zaire ebolavirus* (ChAd3-EBO-Z) and Modified Vaccinia Ankara- vectored Multivalent Filovirus (MVA-BN®-Filo) Vaccine Candidates

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STATEMENT OF COMPLIANCE

The study trial will be carried out in accordance with Good Clinical Practice (GCP) and as required by the following:

- United States Code of Federal Regulations (CFR) 45 CFR Part 46: Protection of Human Subjects
- Food and Drug Administration (FDA) Regulations, as applicable: 21 CFR Part 50 (Protection of Human Subjects), 21 CFR Part 54 (Financial Disclosure by Clinical Investigators), 21 CFR Part 56 (Institutional Review Boards), 21 CFR Part 11, and 21 CFR Part 312 (Investigational New Drug Application [NDA]), 21 CFR 812 (Investigational Device Exemptions [IDE])
- International Conference on Harmonisation: Good Clinical Practice (ICH E6); 62 Federal Register 25691 (1997); and future revisions
- Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, Report of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research
- National Institutes of Health (NIH) Office of Extramural Research, Research Involving Human Subjects, as applicable
- National Institute of Allergy and Infectious Diseases (NIAID) Clinical Terms of Award, as applicable
- Applicable Federal, State, and Local Regulations and Guidance

SIGNATURE PAGE

The signature below provides the necessary assurance that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH E6 Good Clinical Practice (GCP) guidelines.

I agree to conduct the study in compliance with GCP and applicable regulatory requirements.

I agree to conduct the study in accordance with the current protocol and will not make changes to the protocol without obtaining the sponsor's approval and Institutional Review Board (IRB)/Independent or Institutional Ethics Committee (IEC) approval, except when necessary to protect the safety, rights, or welfare of subjects.

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Title

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LIST OF ABBREVIATIONS

Ad	Adenovirus
ADCC	Antibody-Dependent Cell-Mediated Cytotoxicity
AE	Adverse Event/Adverse Experience
ALT	Alanine Transaminase
ANC	Absolute Neutrophil Count
β-HCG	Beta-human chorionic gonadotropin
BMI	Body Mass Index
BP	Blood Pressure
Bpm	Beats per Minute
BUN	Blood Urea Nitrogen
CMS	Clinical Materials Services
CFR	Code of Federal Regulations
ChAd3	Chimpanzee adenovirus Type 3
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CSR	Clinical Study Report
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
EBOV	*Zaire ebolavirus*
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act
FWA	Federal Wide Assurance

GCP	Good Clinical Practice
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C
Hgb	Hemoglobin
HgbA1C	Hemoglobin A1C
HIV	Human Immunodeficiency Virus
HRSA	Health Resources and Services Administration
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
IU	Infectious Units
MedDRA®	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NDA	New Drug Application
NEJM	New England Journal of Medicine
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PHI	Protected Health Information
PI	Principal Investigator

PK	Pharmacokinetics
PT	Prothrombin Time
PTT	Partial thromboplastin Time
PU	Particle Units
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
US	United States
V	Visit
WBC	White Blood Cell
WHO	World Health Organization

PROTOCOL SUMMARY

Title:	A Systems Biology Phase 1 Evaluation of the Safety, Reactogenicity, and Immunogenicity of Chimpanzee Adenovirus Type 3- vectored *Zaire ebolavirus* (ChAd3-EBO-Z) and Modified Vaccinia Ankara-vectored Multivalent Filovirus (MVA-BN®-Filo) Vaccine Candidates
Phase:	1
Population:	60 evaluable males and non-pregnant females, 18-45 years old, inclusive, who are in good health and meet all eligibility criteria
Number of Sites:	1
Study Duration:	Approximately 15 months
Subject Participation Duration:	Approximately 6 months
Description of Agent or Intervention:	*Zaire ebolavirus* (ChAd3-EBO-Z) vaccine, consisting of a recombinant, replication-defective chimpanzee adenovirus (Ad) type 3 (ChAd3)-derived vector expressing the *Zaire ebolavirus* glycoprotein (GP), will be administered by intramuscular (IM) injection in a single dose of 2 x 10^{11} vp virus particles) on Day 1. Booster vaccination will take place on Day 8 for all groups (group 1: placebo, group 2: ChAd3-EBO-Z at dose of 2 x 10^{11} vp {homologous}, group 3: replication defective MVA-BN®-Filo 1 x 10^{8} Infectious Units (IU) per dose vaccine {heterologous} delivered by IM injection). The placebo will be normal saline (0.9% Sodium Chloride, USP).
Objectives:	Primary: • Assess the safety and reactogenicity of study products by study group when administered IM to healthy adults. Secondary: • Assess the antibody response to *Zaire ebolavirus* (EBOV) glycoprotein (GP) by study group. Exploratory: • Assess the EBOV neutralizing antibody titers by study group.

- Assess antibody functionality by antibody-dependent cell-mediated cytotoxicity (ADCC) responses by study group.
- Assess activation of CD4+ and CD8+ T cells in multiple cell subpopulations by study group.
- Assess inflammation via cytokine secretion and C-reactive protein (CRP) by study group.
- Assess EBOV GP-specific T cell responses by study group.
- Assess ChAd3-specific T cell responses by study group.
- Assess plasmablast frequencies by study group.
- Assess B-cell receptor (BCR) repertoire by study group.
- Assess and characterize change in gene expression in PBMCs by study group.
- Identify gene expression changes associated with adaptive humoral and/or cellular immune responses.

Outcome Measures:

Primary:

- Frequency and severity of solicited local and systemic reactogenicity from the time of study vaccine administration through 7 days post-vaccination.
- Frequency and severity of vaccine-related unsolicited adverse events (AEs) from the first study vaccination through 28 days post second vaccination.
- Frequency and severity of clinical safety laboratory AEs from the time of first study vaccination through approximately 28 days after the second vaccination.
- Frequency of Serious Adverse Events (SAEs) and vaccine-related Medically Attended Adverse Events (MAAEs) through 6 months post first vaccination.

Secondary:

- Seroconversion defined as anti-EBOV GP ELISA titer > 50 if baseline (Day 1) titer $\leq$ 50 or fold rise $\geq$ 4 as compared to baseline if baseline titer > 50 on Day 8, 15, 22, 29 and 36.

- Geometric mean titer (GMT) on Day 1, 8, 15, 22, 29 and 36 as measured by anti-EBOV GP ELISA.
- Geometric mean fold rise (GMFR) in titer on Day 8, 15, 22, 29 and 36 compared to baseline (Day 1) as measured by anti-EBOV GP ELISA.

Exploratory:

- GMT of neutralizing antibodies to EBOV GP at Day 1, 8 and 15 as measured by EBOV GP pseudovirion neutralization assay.
- GMFR in titer on Day 8 and 15 compared to baseline (Day 1) as measured by EBOV GP pseudovirion neutralization assay.
- Change in the percentage of killed cells as measured by ADCC on Day 8, 15, 22, 29, and 36 compared to baseline (Day 1).
- Change in percentage of activated CD4+ and CD8+ T cells for each cell subpopulation on Day 8 and 15 compared to baseline (Day 1) as measured by flow cytometry.
- Change in cytokine concentration and CRP on Day 2, 3, 4, 6, 8, 9 and 15 compared to baseline (Day 1) as measured by Luminex assay and clinical laboratory CRP assay, respectively.
- Change in percentage of EBOV GP-specific cytokine secreting CD4+ and CD8+ T cells on Day 8, 15, 22, and 36 compared to baseline (Day 1) as measured by intracellular cytokine staining (ICS).
- Change in percentage of EBOV GP-specific CD4+ and CD8+ T cells on Day 8 and 15 as compared to baseline (Day 1) measured by tetramer staining for predominant human leukocyte antigen (HLA) types.
- Change in percentage of ChAd3-specific cytokine secreting CD4+ and CD8+ T cells on Day 8, 15, 22 and 36 compared to baseline (Day 1) as measured by ICS.
- Change in percentage of CD19+CD27+CD38+ plasmablasts on Day 8 and 15 compared to baseline (Day 1) as measured by flow cytometry.
- Change in B-cell repertoire (BCR) on Day 8 and 15 as compared to baseline (Day 1) from sorted CD19+ CD27+ CD38+

plasmablasts as measured by immunoglobulin heavy chain sequencing.

- Characterization of antigen-specific BCR as measured by yeast display on day 36.
- Change in gene expression in PBMCs on Day 2, 3, 4, 6, 8, 9, and 15 compared to baseline (Day 1) as measured by RNA-Seq.
- Identification of gene expression changes on Day 2, 3, 4, 6, 8, 9, and 15 associated with each subject's peak change in percentage of EBOV GP-specific cytokine secreting CD4+ and CD8+ T cells across Day 15, 22, and 36.
- Identification of gene expression changes on Day 2, 3, 4, 6, 8, 9, and 15 associated with each subject's peak anti-EBOV GP ELISA titer across Day 15, 22, 29, and 36.
- Identification of gene expression changes on Day 2, 3, 4, 6, 8, 9, and 15 associated with each subject's EBOV neutralizing antibody titer at Day 15.
- Identification of gene expression changes on Day 2, 3, 4, 6, 8, 9, and 15 associated with each subject's peak percentage of killed cells as measured by ADCC across Day 15, 22, 29, and 36.

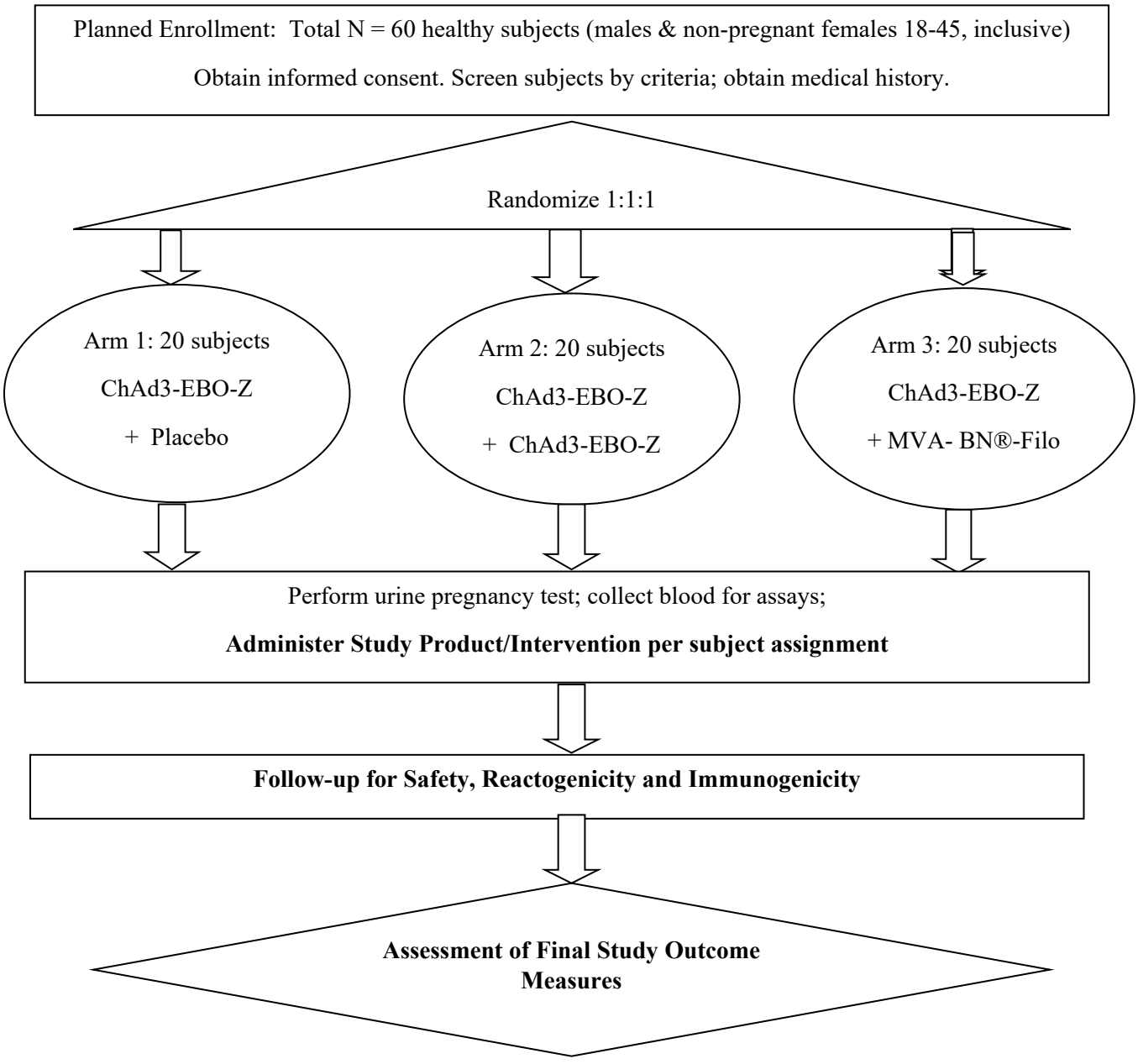
Description of Study Design:

This is a Phase 1, randomized, double-blind trial of 60 evaluable males and non-pregnant females, 18-45 years old, inclusive, who are in good health, meet all eligibility criteria, and do not meet any exclusion criteria. This trial is designed to assess the safety, reactogenicity and immunogenicity of a chimpanzee adenovirus vector expressing *Zaire ebolavirus* glycoprotein (ChAd3-EBO-Z) vaccine with or without MVA- BN®-Filo boost for the prevention of Ebola virus disease. ChAd3-EBO-Z will be administered intramuscularly on Day 1 followed by either placebo (Group 1), ChAd3-EBO-Z (Group 2), or MVA- BN®-Filo (Group 3) at Day 8.

Potential subjects will be screened by history, physical exam, ECG, vital signs, height and weight, and clinical laboratory tests. A urinalysis will be done for urine protein and drug screening for opiates. Potential female subjects of childbearing potential will have a serum pregnancy test. In addition, potential subjects will be screened for Human Immunodeficiency Virus (HIV) type 1 & 2 antibody, Hepatitis C Virus (HCV) antibody, and Surface Antigen for Hepatitis B Virus (HBsAg) prior to enrollment.

	Subjects will be randomized in double-blind fashion per the schedule outlined in Section 4, Table 1. Reactogenicity will be measured by the occurrence of solicited injection site and systemic AEs. Subjects will maintain a memory aid through 7 days post each vaccination, recording temperature, solicited local and systemic symptoms. Unsolicited non-serious adverse events (AEs) will be collected from the time of first study vaccination through approximately 28 days after the second study vaccination. Serious AEs (SAEs) and MAAEs occurring from the time of the first study vaccination through approximately 6 months after the first study vaccination will be collected. Clinical safety labs will be collected on Day 1, 8, 15, 29, and 36; if labs are abnormal, they will be assessed, documented, reported, and followed.until the lab returns to the subject's baseline value or has stabilized. Immunogenicity testing will be performed as outlined in Section 8.2.2. The duration of this trial for each subject will be approximately 6 months.
Estimated Time to Complete Enrollment:	Approximately 5 months

Figure 1: Schematic of Study Design



1 KEY ROLES

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1. Background Information

Ebola viruses are RNA viruses in the family Filoviridae. Ebola viruses have been associated with sporadic outbreaks characterized by severe hemorrhagic disease and high case fatality ratios. *Zaire ebolavirus* (EBOV) and *Sudan ebolavirus* (SUDV) were both identified in 1976 during two simultaneous outbreaks in Zaire (now Democratic Republic of the Congo) and Sudan, respectively[1]. *Taï Forest ebolavirus* (TAFV) arose in Côte d'Ivoire in 1994 and was noted to be distinct from both EBOV and SUDV[2]. Since 1994, outbreaks of Ebola virus disease (EVD) have occurred almost yearly, with the Democratic Republic of Congo being the most frequently affected. *Bundibugyo ebolavirus* (BDBV) is the most recently described member of the genus, identified in Uganda in 2007[2]. A fifth member of the genus, *Reston ebolavirus*, was described in 1990 but is not known to cause human disease[3]. The natural reservoir of the related filovirus Marburg virus (MARV) has been identified as the Egyptian fruit bat[4,5]. The natural reservoir for Ebola viruses is less certain, although fruit bats are likely involved in transmission events to non-human primates and to humans. After an incubation period ranging from 2-21 days, Ebola-associated EVD manifests initially with nonspecific symptoms such as fever, headaches, muscle pain, joint pain, general malaise and gastrointestinal symptoms and in a minority of cases involve hemorrhagic complications[6,7]. One of the most concerning aspects of filovirus outbreaks in humans is the high mortality rate observed. Case fatality rates for EVD measured using World Health Organization (WHO) data prior to 2015 revealed on overall rate of 65.4%, which ranged by species from 76% for EBOV to 55% for SUDV and 37% for BDBV[8]. The case fatality rate tends to be highest in the earliest days of an outbreak due to reporting bias of severe cases. The availability of intensive medical support may also influence the outcome, since the case fatality rate was substantially lower among repatriated US and European patients during the 2014 outbreak of EBOV[9]. While it may be postulated that the experimental clinical interventions that these patients received contributed to their outcomes, the same phenomenon was seen in 1967 in the Marburg outbreak[10] where 31 patients were cared for using only modern supportive care measures including dialysis; the case fatality rate was 22%.

Development of therapeutics and of preventive vaccines against filoviruses is a high global public health priority. The importance of this effort was greatly magnified by the scope of the 2014-2016 West African outbreak of EBOV. The outbreak began in Guinea with an index case of a 2-year-old child who died from EVD in December 2013[11]. The outbreak subsequently became the largest EVD outbreak in history, involving predominantly Guinea, Sierra Leone, and Liberia. According to the WHO, this outbreak involved 28,610 cases and 11,308 deaths (as of March 30, 2016) . Importantly, healthcare workers were disproportionally affected and this resulted in further challenges in the care of EVD patients. Imported cases in Western countries

stimulated additional attention to the epidemic and illustrated the potential for global impact beyond the affected areas in Africa. The extent of the epidemic and the lack of proven therapies and vaccines made further research on Ebola an urgent priority.

Vaccines against Ebola viruses and MARV are under development by a number of academic investigators and manufacturers[12,13]. Developing a safe and effective vaccine against filoviruses is likely to require a greater understanding of the nature of protective immunity against these agents. Animal data support the importance of antibody-mediated protection in studies with DNA prime-adenovirus boost, Ebola virus-like particles (VLPs), vesicular stomatitis virus (VSV)-based vaccines, and parainfluenza virus-based vaccines[14]. In contrast, a recombinant adenovirus serotype 5 (Ad5) EBOV vaccine protected macaques from infection primarily through the activity of CD8+ T cell responses[15]. Acute protection from EBOV in macaques was also elicited by a chimpanzee Ad3 vector EBOV vaccine, and protection correlated with antibody responses, while long-term protection in this study required functional CD8+ T cell responses and was enhanced with a modified vaccinia Ankara (MVA) boost[16]. Therefore, there is currently evidence supporting both antibody-mediated protection and cell-mediated protection against filoviruses. Our understanding of immune correlates of protection is limited largely to animal models, as the sporadic nature of Ebola outbreaks offers limited opportunities for examining protection in humans. It is notable that in the intensely studied patients cared for at Emory University in 2014 the resolution of viremia correlated with the presence of activated CD8 T cells and this occurred prior to the presence of detectable IgG antibody response[17].

2.1.1 Public Readiness and Emergency Preparedness

This protocol, the ChAd3-EBO-Z vaccine manufactured by GSK and the MVA-BN®-Filo vaccine manufactured by Bavarian Nordic, are covered under the Public Readiness and Emergency Preparedness Act (PREP Act). Under the PREP Act, covered persons are immune from liability actions brought from the administration or use of a covered countermeasure that is the subject of a declaration. The PREP Act provides immunity for covered persons (such as manufacturers, distributers, program planners and other qualified persons who prescribe, administer or dispense the study vaccine) from tort liability, unless the injury was caused by willful misconduct.

The PREP Act also authorized a “Covered Countermeasures Process Fund” to provide compensation to eligible individuals who suffer specified injuries from administration or use of a countermeasure pursuant to the declaration. Any requests for compensation must be filed within one year of administration or use of the countermeasure. Requests would go to the HRSA (Health Resources and Services Administration) Preparedness Countermeasures Injury Compensation Program (http://www.hrsa.gov/cicp/). Compensation may then be available for medical benefits, lost wages and death benefits to eligible individuals for specified injuries in

accordance with regulations published by the Secretary. Eligibility for compensation and the injuries for which compensation may be available are further defined by regulation.

An individual who suffers a serious physical injury or death from administration and use of the study vaccine must first seek compensation from the Covered Countermeasures Process Fund. A serious physical injury means an injury that is life threatening, results in, or requires medical or surgical intervention to prevent, permanent impairment of a body function or permanent damage to body structure. Any compensation will be reduced by public or private insurance or worker's compensation available to the injured individual.

If no funds have been appropriated to the compensation program, the Secretary does not make a final determination on the individual's request within 240 days, or if the individual decides not to accept the compensation, the injured individual or his representative may pursue a tort claim in the United States District Court for the District of Columbia, but only if the claim involves willful misconduct, is pled with particularity required under the PREP Act, verified, and accompanied by an affidavit by a physician who did not treat the individual and certified medical records. Any award is reduced by any public or private insurance or worker's compensation available to the injured individual. Awards for non-economic damages, such as pain, suffering, physical impairment, mental anguish, and loss of consortium are also limited. If the individual accepts compensation, or if there is no willful misconduct, then the individual does not have a tort claim that can be filed in a United States Federal or a State court.

2.2. Rationale

Development of a vaccine to protect from infection or disease caused by EBOV is a national priority. No licensed vaccines or therapeutics are currently available for EVD. The extensive EBOV outbreak in West Africa during 2014-2016 illustrates the urgent need for preventive measures against Ebola. This Phase 1 vaccine trial will provide a unique opportunity to define innate immune responses elicited by a promising chimpanzee adenovirus vector expressing *Zaire ebolavirus* glycoprotein (EBOV GP). This trial offers a chance to compare responses elicited by homologous vs. heterologous boosting using a rapid vaccination schedule. The study may also have applicability beyond Ebola as the vaccine vectors (ChAd and MVA) are being used to develop vaccines against other pathogens.

The Vaccine Research Center of the National Institute of Allergy and Infectious Diseases (VRC/ NIAID), National Institutes of Health (NIH) in the United States of America (US) has been developing replication-defective recombinant chimpanzee adenovirus Type3-vectored monovalent and bivalent filovirus vaccine candidates. These include monovalent ChAd3-EBO-Z for EBOV and ChAd3-MARV for MARV, as well as bivalent ChAD3-EBO (EBOV + SUDV). ChAd3 recombinants were developed using the Okairos adenovirus vaccine platform technology acquired by GlaxoSmithKline (GSK) in May 2013[18]. The DNA

fragment inserted in ChAd3-EBO-Z encodes the wild-type (WT) glycoprotein (GP) from the species EBOV. GP is expressed on the surface of the virus and is critical for attachment to host cells and catalysis of membrane fusion. GP is the major target for neutralizing antibody responses against Ebola viruses and contains cytotoxic T lymphocyte and T helper epitopes that contribute to protection in animal models[15,16].

Pre-clinical immunogenicity and efficacy studies were conducted with the investigational ChAd3-EBO-Z vaccine and with the investigational ChAd3-EBO (bivalent) vaccine in cynomolgus macaques. Overall, it was shown that a single intramuscular (IM) dose of $1x10^9$ or $1x10^{10}$pu of either monovalent or bivalent vaccine elicited anti-GP antibodies and antigen-specific CD4+ and CD8+ T-cell responses. 100% of macaques receiving a single IM dose of 10^{10} - 10^{11} pu of the bivalent ChAd3-EBO were protected from against EBOV or SUDV challenge 4-5 weeks after vaccination, whereas a dose of $1x10^9$ pu provided protection in half of the vaccinated cynomolgus macaques. ChAd3-EBO-Z given at 10^{10} or 10^{11} pu was 100% protective against EBOV challenge at 4-5 weeks post-vaccination. Ten months after vaccination, the humoral and the cellular immune response had declined, and protection against EBOV challenge was observed in half of the animals who had received a dose of 10^{11} pu and in none of the animals who had received a dose of $1x10^{10}$ pu[16]. Durability of protection was extended to ten months in macaques receiving ChAd3-EBO priming followed by bivalent (EBOV, SUDV GP) MVA boosting[16].

Phase 1 and Phase 2 trials support the use of replication-defective adenovirus approaches to stimulate Ebola-specific immune responses in humans. VRC 205 evaluated two replication-defective recombinant Ad5 vectors that encoded GP from the species EBOV and SUDV, respectively. Antigen-specific humoral and cellular responses were observed following a single intramuscular injection and the vaccine was generally safe and well-tolerated[19]. VRC 207 evaluated bivalent ChAd3-EBO in healthy adults. At 4 weeks after vaccination, vaccine-induced antibodies to EBOV GP were present in 90% of individuals receiving 2 x 10^{10} pu dose and 100% of individuals receiving a 2 x 10^{11} pu dose. GP-specific CD8 T cell responses were detected in 20% of individuals by week 4 in the lower dose group and 70% of individuals receiving the higher dose. Overall the study supported the safety and immunogenicity of a single dose of ChAd3-EBO in humans[20]. ChAd3-EBO-Z is a monovalent vaccine that has also been evaluated in a series of trials (a total of 15 clinical trials enrolling approximately 5,000 subjects) in U.S. and international sites, either with or without MVA boost. In a phase 1 study performed in the United Kingdom, a single dose of ChAd3-EBO-Z was administered to 60 healthy adult volunteers. No significant safety concerns with ChAd3-EBO-Z were identified in this study. Boosting with MVA- BN®-Filo (described below) significantly enhanced GP-specific antibody and CD8+ T cell responses that were elicited by ChAd3-EBO-Z, and antibodies remained higher in the boosted subjects 6 months after vaccination[21]. A phase 1 trial performed in the United States and in Mali during the West African Ebola epidemic also

evaluated ChAd3-EBO-Z prime and MVA- BN®-Filo boost. Safety was again demonstrated, and a 1 x 10^{11} pu dose of ChAd3-EBO-Z was identified as sufficiently immunogenic to move forward as a potential means of interrupting transmission in the African setting. Boosting with MVA- BN®-Filo increased the magnitude and extended the duration of antibody responses and anamnestic polyfunctional CD4+ and CD8+ T cell responses[22]. A phase 2 trial performed in Liberia enrolled 500 adults who received a single dose of ChAd3-EBO-Z. Side effects were not severe, and anti-GP antibodies developed in 70% of participants by 1 month and remained detectable at 12 months in 63.5% of participants[23].

The MVA multi-filovirus vaccine from Bavarian Nordic (MVA-mBN226B, referred to subsequently as MVA- BN®-Filo) encodes the GPs of EBOV, SUDV, and MARV, together with the nucleoprotein (NP) of TAFV. Since GP is the surface glycoprotein, it is the natural target of neutralizing antibodies, which often (but not always) correlate with protection in animal models[24]. The major targets of cellular immunity in humans are the NP followed by the GP protein[17]. Therefore, the potential with this vaccine is to elicit protective antibodies against three major filoviruses as well as potentially cross-protective cellular immunity[25]. MVA is an attenuated poxvirus derived by more than 500 serial passages in chicken embryo fibroblast cells and contains well-studied internal deletions that account for its low virulence. Phase 3 trial data with the Bavarian Nordic MVA backbone demonstrates immunogenicity and a reasonable side effect profile in Phase 3 clinical trials. The clinical safety profile of MVA-based recombinant vaccines has been established in numerous Phase 1 and Phase 2 trials[26,27]. Immunogenicity studies of MVA multi-filovirus vaccine in nonhuman primates (NHPs) demonstrated substantial binding and neutralizing antibodies against EBOV, SUDV, and MARV. Some protection in MVA-BN®-Filo vaccine recipient NHPs was observed. However, the use of a heterologous prime-boost regimen with recombinant adenovirus-based vaccines resulted in much more complete levels of protection. In these NHP studies, protection was observed regardless of the order of the prime or boost. These results led to a series of trials in humans combining replication-defective adenovirus vectors and MVA-BN®-Filo. Under Janssen's sponsorship, three Phase 1 clinical trials have completed recruitment and data for the clinical study reports (CSR) are being analyzed; one Phase 1, three Phase 2 and three Phase 3 clinical trials are ongoing and actively recruiting. In addition, a first-in-human Phase 1 clinical trial of a combination regimen of the multivalent Ad26.Filo as prime in combination with MVA-BN®-Filo as boost vaccination is currently ongoing. In the ongoing clinical trials, MVA-BN®-Filo is administered as prime or as boost in different heterologous prime-boost regimens in combination with Ad26.ZEBOV or Ad26.Filo (multivalent). The current experience amounts to more than 1,700 subjects that have been enrolled in the completed and ongoing clinical trials and no safety concerns have arisen from the ongoing clinical program.

The current clinical study has two major objectives. First, to further characterize and better understand whether innate immune responses as measured at early time points following

vaccination with ChAd-EBO-Z are associated with late endpoints such as neutralizing antibody titers, T cell responses, and potentially protection from EVD. Second, to evaluate the effect of homologous or heterologous boosting, with a short interval employed between prime and boost vaccination. The advantage of the short interval is that it minimizes the risk of post-immunization anti-vector (ChAd3) antibodies interfering with the boost response, and differs from other studies such as 14-day intervals as tested in NCT02451891 in which the adaptive response had already evolved significantly. A short interval also has the potential advantage of accelerating the development of immune responses for those at risk in an outbreak setting or for aid workers deployed to outbreak areas. This trial will employ systems biology tools[28], in addition to immunology and clinical chemistry methods, to explore whether the kinetics, magnitude and profile of host responses following first and second vaccination can provide insights into potential mechanisms of protection and the differences between protective and non-protective states.

2.3. Potential Risks and Benefits

2.3.1. Potential Risks

The potential risks to subjects due to participation in the study are those associated with venipuncture, intramuscular injection (IM), and possible reactions to the experimental vaccine(s) or placebo. There may be potential risk related to breach of confidentiality, as well as other unknown risks, discomforts, or side effects.

Venipuncture causes transient discomfort and may result in fainting. Fainting is usually transient and managed by having the subject lie down and elevate his/her legs. Bruising at the site of the venipuncture may occur but may be prevented or lessened by applying pressure to the site for several minutes. Venipuncture may also cause infection. The use of aseptic (sterile) technique will make infection at the site where blood will be drawn extremely unlikely.

Intramuscular injection may cause transient discomfort and fainting. Giving an IM injection may predispose a subject to infection. However, the use of sterile technique will make an infection at the injection site extremely unlikely.

Acute and potentially life-threatening allergic reactions (e.g., anaphylaxis) are also possible as a result of vaccination. Very rarely there can be a serious allergic reaction. These reactions can manifest as skin rash (hives); swelling around the mouth, throat, or eyes (angioedema); difficulty breathing (bronchospasm), a fast pulse (tachycardia), or drop in blood pressure (hypotension). If these reactions occur, they can be usually stopped by the administration of emergency medications by the study personnel. As with any vaccine or medication, there is a very small chance of a fatal reaction (death), although researchers do not expect this to occur.

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 vaccine induced seropositivity (VISP). These antibodies may be detected in Ebola serologic tests, causing the test to appear positive even in the absence of actual Ebola virus infection. VISP may become evident during the study, or after the study has been completed. Subjects should not donate blood for 3 months after receiving the second study vaccine.

It is unknown if MVA-BN®-Filo or ChAd3-EBO-Z vaccines pose risk to egg, conception, pregnancy, an unborn child, or a breastfeeding infant. Women of childbearing potential who are not sterilized via tubal ligation, bilateral oophorectomy, hysterectomy, successful Essure® placement (permanent, non-surgical, non-hormonal sterilization) with history of documented radiological confirmation test at least 90 days after the procedure (or with use of another birth control method if history of confirmation test not confirmed, still menstruating, or < 1 year of the last menses if menopausal must agree to practice effective contraception for a minimum of 30 days prior to study product exposure and agree to practice effective contraception for the duration of study product exposure, including 90 days after the second study vaccination. If a female subject becomes pregnant while participating in this trial, we will ask permission to follow-up about her health through pregnancy outcome and the health of her baby.

Subjects will be asked to provide protected health information (PHI). All attempts will be made to keep this information within the limits of the law. However, there is a chance that unauthorized persons will see the subjects' PHI. All records will be kept in a locked file cabinet or maintained in a locked room at the Cincinnati Children's Hospital Medical Center (CCHMC) Vaccine and Treatment Evaluation Unit (VTEU) site. Electronic files will be password protected. Only people who are involved in the conduct, oversight, monitoring, or auditing or this study will be allowed access to PHI that is collected. Any publications from this study will not use information that will identify subjects by name. Organizations that may inspect and/or copy research records maintained at the participating VTEU sites for quality assurance (QA) and data analysis include groups such as the National Institute of Allergy and Infectious Diseases (NIAID) and the FDA.

The MVA-BN®-Filo and ChAd3-EBO-Z vaccines are currently under investigation for use in humans.Chimpanzee adenovirus Type 3 ('ChAd3', or VRC designation 'cAd3') is a subgroup C adenovirus with properties similar to those of human Ad5. Serological studies showed a low seroprevalence in human sera for antibodies to ChAd3 and when present, antibody titers were low[29]. In addition, Ad5 pre-existing immunity did not appear to cross-react with ChAd3 in mice[30].

ChAd3-EBO-Z

Recombinant, replication-defective ChAd3-EBO-Z-based vaccines have been shown capable of inducing an immune response comparable to Ad5-based vaccines in mice[30,31]. In two Phase 1

studies with an investigational recombinant ChAd3-vectored hepatitis C virus (HCV) vaccine, at total of 65 healthy adults were vaccinated by IM injection with doses up to $7.5x10^{10}$ pu [studies HCV 001 and HCV 003; ClinicalTrials.gov identifiers NCT01070407 and NCT01296451, respectively]. Several prime-boost schedules were evaluated with 4 -11 subjects per group. Mostly mild, self-limiting local and systemic reactogenicity was observed which was dose-dependent but did not differ significantly between priming and boosting and overall, these vaccines were assessed as having an acceptable safety profile. The vaccinations induced HCV-specific immunity with broad specificity that was sustained for at least a year after the boost with heterologous adenoviral vector[32,33].

Since the beginning of ChAd3-EBO-Z's development (IBD 09 September 2014), it has been administered to 3421 healthy study participants from the age of 1 and above in doses up to $1x10^{11}$ pu. Note that 1 x 10^{11} pu as used in these studies is equivalent to 2 x 10^{11} vp. The present protocol uses the vp designation. The summary below was generated from data available through 08 September 2017, from subjects enrolled in Phase 1 and Phase 2 studies. For more detailed safety information please refer to the product IB.

Safety in Phase I Studies:

For dose selection, four clinical Phase 1 studies assessing the investigational monovalent ChAd3-EBO-Z vaccine have been completed. In these studies, 271 healthy adults received one dose of ChAd3-EBO-Z vaccine at one of four different doses ranging from $1x10^{10}$ pu to $1x10^{11}$ pu. In one of these studies (study VRC207), 2 different doses of the bivalent investigational ChAd3-EBO vaccine ($2x10^{10}$ pu and $2x10^{11}$ pu) were assessed as well, and in 2 of the studies (EBL01 and EBL03)[22] heterologous boosting with the multivalent MVA-vectored vaccine MVA-BN®-Filo (containing an insert for EBOV GP, as well as the SUDV GP, the Musoke strain MARV glycoprotein, and a nucleoprotein from the TAFV) were assessed.

Published data from VRC 207 reported mild to moderate reactogenicity and no SAEs. An artificially prolonged aPTT was seen in three volunteers but was found to be an effect on the laboratory assay and not clinically significant [20]. Published data from EBL01 reported mostly mild reactogenicity and no SAEs [21]. Published data from the Mali1000 (EBL03) reported solicited AEs observed for 7 days following booster vaccination with MVA-BN®-Filo included local pain and tenderness, fever, fatigue, myalgia, arthralgia, headache and chills. There were no SAEs following boost with MVA-BN®-Filo or saline placebo. Serological responses of the MVA-BN®-Filo booster participants showed that the GMT 28 days after boosting was significantly higher than that 28 days after priming for the 27 participants boosted with MVA-BN®-Filo[22].

No Grade 3 local solicited AEs were reported in any of the dose groups in any of the initial Phase 1 trials that have evaluated ChAd3-EBO-Z. Pain was the most frequently reported Grade 2

local AE reported with a variable range by trial and not clear trend for an increase by dose. Pain was also the most frequently reported Grade 1 local AE with a variable range by trial and increasing lower limit by dose [0-10.5%] with $1x10^{10}$ pu, [0-10.0%] with $2.5x10^{10}$ pu, [0-15.0%] with $5x10^{10}$ pu, and [0-9.1%] with $1x10^{11}$ pu. 25.0% of the subjects in the placebo group in the study ChAd3-EBOZ Lau (Lausanne) (only trial with a placebo control group) reported Grade 1 pain.

Grade 3 systemic solicited AEs were reported sporadically with $2.5x10^{10}$ and $5x10^{10}$ pu, and none were reported with 1 $x10^{11}$ pu. Systemic AEs occurred mostly within 24 hours after vaccination and were generally self-limiting within 24 hours. Overall, a trend for an increase in systemic AEs by increasing dose was observed: $1x10^{10}$ pu (70.0% in VRC207 and 50.0% in EBL03) and $1x10^{11}$ pu (80.0% in VRC207 and 63.6% in EBL03) across trials.

Fever within 7 days post-vaccination was observed in 5% of the subjects in the control group, in none of the subjects who received the $1x10^{10}$ pu dose, in 14.2% of the subjects who received $2.5x10^{10}$ pu, in 19.2% of the subjects who received $5x10^{10}$ pu and in 38.1% of the subjects in the $1x10^{11}$ pu groups. The only Grade 3 fever occurred with $5x10^{10}$ pu in the study ChAd3-EBOZ Lau. The onset of fever was mostly on Day 0-1 (except for 6 cases on Day 3-6) and fever was mostly self-limiting within 1 day. There was a clear dose-dependent increase in fever (any grade).

Transient non-clinically significant lymphopenia, neutropenia and anemia were observed within the first 28 days post vaccination with all doses (except neutropenia with $1x10^{11}$ pu) across all trials (including lymphopenia and neutropenia in the placebo control group in the study ChAd3-EBOZ Lau [only trial with a placebo control group]). The majority of these events were Grade 1. There is no evidence suggesting that the magnitude of these non-clinically significant changes increases with increasing vaccine dose.

Seven cases of transient platelet count drops met as per protocol criteria for thrombocytopenia ($<150x10^9$/L), 4 in subjects who received $2.5x10^{10}$ pu and 3 in subjects who received $5x10^{10}$ pu. No clinical signs or symptoms suggestive of increased tendency for bleeding were reported. No thrombocytopenia was reported with $1x10^{10}$ and $1x10^{11}$ pu. Transient non-clinically significant drops in platelet count within 7 and within 28 days post-vaccination not meeting criteria for thrombocytopenia seem to be induced by vaccination (‘real’ phenomenon) i.e. seen consistently across trials and seen less frequently with in the placebo group included in the study ChAd3-EBOZ Lau.

No significant related unsolicited AEs were reported with any of the doses in any of the trials. No Suspected Unexpected Serious Adverse Reactions were reported. The only SAE reported to date (peritoneal tuberculosis 14 days post vaccination) is considered unrelated to vaccination.

Safety in Phase II Studies:

Two phase II studies of ChAd3-EBO-Z have been performed, EBOLA Z CHAD3-004 and EBOLA Z CHAD3-005, encompassing both pediatric and adult age groups. Grade 3 local solicited adverse events (AEs) were reported in 0.4% adult and 1.3% pediatric subjects in the EBO-Z group. Pain was the most frequently reported solicited local AE. Pain was reported by 47.6% in adult and 31.2% in pediatric subjects (ranging from 31.0% to 54.5%) in the EBO-Z group and 7.6% in adults and 20.0% subjects in the control group in pediatrics (ranging from 14.0% to 23.2%).

Systemic AEs occurred mostly within 24 hours after vaccination and were generally self-limiting within 24 hours. Grade 3 **systemic solicited AEs** were reported by 2.8% in adult and 51.3% in pediatric subjects in EBO-Z group, and 0.8% in adult and 16.7% in pediatric subjects in the control group. In adults, the most commonly reported mild to moderate systemic AEs were headache and fatigue; 46.1% and 38.0% subjects in the EBO-Z group and 18.1% and 12.5% subjects in control group, respectively.

Thrombocyte count drops from baseline levels (the majority of them occurring within the normal range) were observed in the EBO-Z and the control group in adult and pediatric subjects without notable differences between groups, and no clinical signs of thrombocytopenia (AESI) were reported within the first 7 days post-vaccination in either of the groups.

Unsolicited AEs were reported by 16.4% of adult and 13.7% of pediatric subjects in the EBO-Z group and 15.8% of adult and 8.0% pediatric subjects in the control group. Grade 3 unsolicited AEs that were considered to be related to the study vaccination by the Investigator were reported by 0.9% subjects in the EBO-Z group and 0.1% subject in the control group in adults and no Grade 3 unsolicited AEs were reported in pediatrics.

Among the adult subjects, **SAEs** were reported by 11 (0.7%) subjects in the EBO-Z group and 18 (1.2%) subjects in the Placebo/ EBO-Z group over the one year follow-up period. No SAE was considered to be related to the study vaccination per the Investigator's judgement.

For complete safety information from phase II trials please refer to the product IB.

<u>MVA-BN® and MVA-BN®-Filo</u>

MVA-BN® and the MVA-BN® based recombinant vaccines including MVA-BN®-Filo are highly attenuated, replication defective strains of vaccinia that are unable to replicate in human cells. Therefore, they cannot be transmitted or cause dispersed vaccinia infection.

BN has performed an extensive nonclinical development program for MVA-BN® demonstrating the safety and efficacy of MVA-BN®. Since BN initiated clinical development of MVA-BN® in

2001, more than 10,500 subjects have been vaccinated with MVA-BN® and MVA-BN® based recombinant vaccines in clinical trials sponsored by BN, Janssen, the NIH and other third-party sponsors. MVA-BN®-Filo vaccine has been studied in test tubes and in animals and has been used in limited numbers of people so far. Safety information was combined from 239 participants who received MVA-BN®-Filo in the first four studies. In general, MVA-BN®-Filo has been shown to be well-tolerated.

Across all clinical trials, no trends for unexpected and/or serious suspected adverse reactions due to the investigational product were detected. MVA-BN® and its recombinant derivatives have been shown to be safe in healthy as well as in immunocompromised populations that are contraindicated to receive conventional, replication competent vaccinia vaccines, e.g., individuals with HIV infection or diagnosed with atopic dermatitis. Moreover, the safety of MVA-BN® was confirmed in an elderly population up to 80 years of age. No difference in the safety profile has been observed between vaccinia-naïve and vaccinia-experienced subjects receiving MVA-BN®.

To date, more than 7,800 subjects have received at least one vaccination with MVA-BN® in completed and ongoing clinical trials. A total of seven serious suspected adverse drug reactions (sarcoidosis, Crohn's disease, transitory ocular muscle paresis, congestive heart failure due to cardiomyopathy, pneumonia and pleurisy, hypersensitivity, and non-ST segment elevation myocardial infarction) have been reported for MVA-BN® so far (7 out of 7,871 vaccinated subjects = 0.09%). All of them have been thoroughly reviewed by BN and the trial specific Data and Safety Monitoring Board (DSMB), who concluded that the continued use of MVA-BN® in a clinical setting presented no special risks to the subjects.

None of the severe adverse reactions associated with replicating VV (vaccinia virus) smallpox vaccines, such as progressive vaccinia, eczema vaccinatum, generalized vaccinia and inadvertent (auto) inoculation have been reported in any of the clinical trials with MVA-BN®. Given that MVA-BN® is replication incompetent in mammalian cells, it can essentially be ruled out that MVA-BN® would induce these side effects associated with replication competent VV[34].

Importantly, MVA-BN® does not increase the risk for developing myo-/pericarditis, in contrast to the older, replication-competent vaccinia based vaccines. Despite close cardiac monitoring, no case of confirmed myocarditis has been reported in the 7,871 subjects vaccinated with the backbone vector MVA-BN® so far. Only 1 possible, albeit doubtful, case of pericarditis (consisting of chest pain only, with no other cardiac findings suggestive of pericarditis) has been observed in the MVA BN clinical trial program. In addition, the safety of MVA-based recombinant vaccines has been confirmed (using doses up to five times higher than used with

MVA-BN®) in more than 900 subjects in completed clinical trials for other products than MVA-BN®-Filo. These trials included children 6 months to 6 years of age and high-risk populations like subjects with HIV infection or suffering from melanoma. For the present study, subjects will be actively screened to exclude preexisting cardiac concerns."

MVA-BN®-Filo vaccine has been studied in animals and has been used in limited numbers of people so far. The ongoing clinical development program, which was initiated in November 2014 is sponsored by Janssen. Under Janssen's sponsorship, three Phase I clinical trials have completed recruitment and data for the clinical study reports are being analyzed; one Phase I, three Phase II and three Phase III clinical trials are ongoing and actively recruiting. In addition, a first-in-human Phase I clinical trial of a combination regimen of the multivalent Ad26.Filo as prime in combination with MVA-BN®-Filo as boost vaccination is currently ongoing. In the ongoing clinical trials, MVA-BN®-Filo is administered as prime or as boost in different heterologous prime-boost regimens in combination with Ad26.ZEBOV or Ad26.Filo (multivalent). The current experience amounts to more than 1,700 subjects that have been enrolled in the completed and ongoing clinical trials and no safety concerns have arisen from the ongoing clinical program.

The preliminary safety profile from a pooled safety data analysis from four Phase 1 studies (VAC52150EBL1001, VAC52150EBL1002, VAC52150EBL1003 and VAC52150EBL1004) showed that the majority of the unsolicited AEs were mild (grade 1) or moderate (grade 2) in severity and all were transient in nature and resolved without sequelae. Two-thirds of the participants reported at least one local site reaction. Three-fifths of the participants reported at least one systemic symptom.The frequency of local and solicited systemic AEs is similar to that seen with other injectable vaccines, with the most frequent being local pain and headache, fatigue and myalgia. Overall, no clinically significant trend in laboratory assays was found. Grade 3 laboratory abnormalities reported included hyperkalemia, hypokalemia, decreased hemoglobin, decreased neutrophils, decreased platelets and proteinuria. These cases were isolated and no common trend was found. One subject in the study VAC52150EBL2001 experienced a serious and very rare condition called "Miller Fisher syndrome". This condition consists of double vision, pain on moving the eye, and difficulty with balance while walking. Miller Fisher syndrome most commonly occurs following a recent infection. The subject experienced these symptoms about a week after suffering from a common cold and fever. The event happened about a month after boost vaccination with either MVA-BN®-Filo or placebo. This subject had to go to the hospital for treatment and has recovered. After an extensive investigation, the event has been considered to be doubtfully related to vaccine and most likely related to the previous common cold.

In conclusion, the available nonclinical and clinical data showed no unexpected findings and no target organ toxicity. MVA-BN® and MVA-BN® based recombinant vaccines, including MVA-BN®-Filo,have demonstrated a very good safety profile combined with the ability to induce

strong immune responses. Furthermore, available data in subjects with contraindications to conventional vaccinia vaccines such as patients with atopic dermatitis (AD) and HIV infection revealed no special risks or safety concerns following MVA-BN® handling and administration.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by US Law. This web site will not include information that can identify subjects. This web site will include a summary of the results in a tabular format including participant flow; demographic and baseline characteristics; primary outcomes, as well as results of any scientifically appropriate statistical tests; and adverse event information.

2.3.2. Known Potential Benefits

There are no known benefits from receiving MVA-BN®-Filo or ChAd3-EBO-Z, but there is the prospect of benefit. Subjects may develop immune responses to filoviruses, but they may not be protected. Society may benefit from the potential development of a new vaccine regimen against filoviruses. In addition, information learned from this study may impact overall understanding of cellular and immunologic processes before and after vaccination and generate other hypotheses that may impact future vaccine development.

3 OBJECTIVES AND OUTCOME MEASURES

3.1. Study Objectives

3.1.1. Primary

- Assess the safety and reactogenicity of study products by study group when administered IM to healthy adults.

3.1.2. Secondary

- Assess the antibody response to Zaire ebolavirus (EBOV) glycoprotein (GP) by study group.

3.1.3. Exploratory (if applicable)

- Assess the EBOV neutralizing antibody titers by study group.
- Assess antibody functionality by antibody-dependent cell-mediated cytotoxicity (ADCC) responses by study group.
- Assess activation of CD4+ and CD8+ T cells in multiple cell subpopulations by study group.
- Assess inflammation via cytokine secretion and C-reactive protein (CRP) by study group.
- Assess EBOV GP-specific T cell responses by study group.
- Assess ChAd3-specific T cell responses by study group.
- Assess plasmablast frequencies by study group.
- Assess B-cell receptor (BCR) repertoire by study group.
- Assess and characterize change in gene expression in PBMCs by study group.
- Identify gene expression changes associated with adaptive humoral and/or cellular immune responses.

3.2. Study Outcome Measures

3.2.1. Primary

- Frequency and severity of solicited local and systemic reactogenicity from the time of study vaccine administration through 7 days post-vaccination.
- Frequency and severity of vaccine-related unsolicited adverse events (AEs) from the first study vaccination through 28 days post second vaccination.

- Frequency and severity of clinical safety laboratory AEs from the time of first study vaccination through approximately 28 days after the second vaccination.
- Frequency of Serious Adverse Events (SAEs) and vaccine-related Medically Attended Adverse Events (MAAEs) through 6 months post first vaccination.

3.2.2. Secondary

- Seroconversion defined as anti-EBOV GP ELISA titer > 50 if baseline (Day 1) titer ≤ 50 or fold rise ≥ 4 as compared to baseline if baseline titer > 50 on Day 8, 15, 22, 29 and 36.
- Geometric mean titer (GMT) on Day 1, 8, 15, 22, 29 and 36 as measured by anti-EBOV GP ELISA.
- Geometric mean fold rise (GMFR) in titer on Day 8, 15, 22, 29 and 36 compared to baseline (Day 1) as measured by anti-EBOV GP ELISA.

3.2.3. Exploratory

- GMT of neutralizing antibodies to EBOV GP at Day 1, 8 and 15 as measured by EBOV GP pseudovirion neutralization assay.
- GMFR in titer on Day 8 and 15 compared to baseline (Day 1) as measured by EBOV GP pseudovirion neutralization assay.
- Change in the percentage of killed cells as measured by ADCC on Day 8, 15, 22, 29, and 36 compared to baseline (Day 1).
- Change in percentage of activated CD4+ and CD8+ T cells for each cell subpopulation on Day 8 and 15 compared to baseline (Day 1) as measured by flow cytometry.
- Change in cytokine concentration and CRP on Day 2, 3, 4, 6, 8, 9 and 15 compared to baseline (Day 1) as measured by Luminex assay and clinical laboratory CRP assay, respectively.
- Change in percentage of EBOV GP-specific cytokine secreting CD4+ and CD8+ T cells on Day 8, 15, 22, and 36 compared to baseline (Day 1) as measured by intracellular cytokine staining (ICS).
- Change in percentage of EBOV GP-specific CD4+ and CD8+ T cells on Day 8 and 15 as compared to baseline (Day 1) measured by tetramer staining for predominant human leukocyte antigen (HLA) types.
- Change in percentage of ChAd3-specific cytokine secreting CD4+ and CD8+ T cells on Day 8, 15, 22 and 36 compared to baseline (Day 1) as measured by ICS.

- Change in percentage of CD19+CD27+CD38+ plasmablasts on Day 8 and 15 compared to baseline (Day 1) as measured by flow cytometry.
- Change in B-cell repertoire (BCR) on Day 8 and 15 as compared to baseline (Day 1) from sorted CD19+ CD27+ CD38+ plasmablasts as measured by immunoglobulin heavy chain sequencing.
- Characterization of antigen-specific BCR as measured by yeast display on day 36.
- Change in gene expression in PBMCs on Day 2, 3, 4, 6, 8, 9, and 15 compared to baseline (Day 1) as measured by RNA-Seq.
- Identification of gene expression changes on Day 2, 3, 4, 6, 8, 9, and 15 associated with each subject's peak change in percentage of EBOV GP-specific cytokine secreting CD4+ and CD8+ T cells across Day 15, 22, and 36.
- Identification of gene expression changes on Day 2, 3, 4, 6, 8, 9, and 15 associated with each subject's peak anti-EBOV GP ELISA titer across Day 15, 22, 29, and 36.
- Identification of gene expression changes on Day 2, 3, 4, 6, 8, 9, and 15 associated with each subject's EBOV neutralizing antibody titer at Day 15.
- Identification of gene expression changes on Day 2, 3, 4, 6, 8, 9, and 15 associated with each subject's peak percentage of killed cells as measured by ADCC across Day 15, 22, 29, and 36.

4 STUDY DESIGN

This is a Phase 1, randomized, double-blind trial of 60 males and non-pregnant females, 18-45 years old, inclusive, who are in good health and meet all eligibility criteria. This trial is designed to assess the safety, reactogenicity and immunogenicity of a *Zaire ebolavirus* vaccine (ChAd3-EBO-Z) for the prevention of EVD. Vaccine will be administered intramuscularly in a single dose regimen on Day 1 followed by a booster dose at Day 8 for all groups (group 1: placebo boost, group 2: ChAd3-EBO-Z boost, group 3: MVA-BN®-Filo boost).

Potential subjects will be screened by history, physical exam, ECG, vital signs, height and weight, and clinical laboratory tests (refer to Appendix A: SUPPLEMENTS/APPENDICES) prior to enrollment.

Subjects will be randomized in double-blind fashion to 1 dose of ChAd3-EBO-Z vaccine at Day 1 followed by a boost at Day 8. Doses will be administered per the schedule outlined in the Table 1 below.

Table 1: Treatment Arms

Treatment Arm	Subjects Enrolled (n)	Day 1	Day 8
1	20	2 x 10^{11} vp ChAd3-EBO-Z	Placebo
2	20	2 x 10^{11} vp ChAd3-EBO-Z	2 x 10^{11} vp ChAd3-EBO-Z
3	20	2 x 10^{11} vp ChAd3-EBO-Z	1 x 10^{8} IU MVA-BN®-Filo
Total	60 subjects		

On vaccination days, all females of childbearing potential will have a urine pregnancy test done. All subjects will have baseline vital signs, targeted physical examination, clinical safety laboratory tests (refer to Appendix A: SUPPLEMENTS/APPENDICES) done, and antibody levels measured. Vaccine or booster will then be administered by intramuscular injection into the deltoid muscle. Subjects and study staff will be blinded to treatment arm. Venous blood will be collected for high resolution HLA typing for Class 1 (A, B, and C locus) testing on Day 1 prior to vaccination.

Reactogenicity will be measured by the occurrence of solicited injection site and systemic AEs. Subjects will maintain a memory aid through 7 days post each vaccination, recording temperature, solicited local and systemic symptoms. Unsolicited non-serious AEs will be collected from the time of first study vaccination through approximately 28 days after the second study vaccination. Serious AEs and MAAEs occurring from the time of the first study vaccination through approximately 6 months after the first study vaccination will be collected. Clinical safety labs will be collected on Day 1, 8, 15, 29, and 36; if labs are abnormal, they will

be assessed, documented, reported, and followed until the lab returns to the subject's baseline value or has stabilized.

Immunogenicity testing will be performed as outlined in Section 8.2.2. Blood will be collected for future exploratory assays as described in Section 8.2.2 and Appendix A: Schedule of Study Procedures and Evaluations. The duration of this trial for each subject will be approximately 6 months.

For additional details on study procedures and evaluations by study visits/days, see Section 7 and Appendix A: Schedule of Study Procedures and Evaluations.

5 STUDY ENROLLMENT AND WITHDRAWAL

Sixty males and non-pregnant females, 18-45 years old, inclusive, who are in good health and meet all eligibility criteria, will be enrolled from a single Vaccine and Treatment Evaluation Unit (VTEU) site. The target population should reflect the community at large at the participating VTEU site. Estimated time to complete enrollment in this trial is approximately 5 months. Information regarding this trial may be provided to potential subjects who have previously participated in vaccine trials conducted at the participating VTEU site. Other forms and/or mechanisms of recruitment may also be used. The local Institutional Review Board (IRB) will approve all materials prior to use.

Subject Inclusion and Exclusion Criteria must be assessed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

No exemptions are granted on Subject Inclusion/Exclusion Criteria in DMID-sponsored studies. Questions about eligibility will be directed toward the DMID Medical Officer.

5.1. Subject Inclusion Criteria

Subjects eligible to participate in this study must meet all of the following inclusion criteria:

1. Provide written informed consent before initiation of any study procedures.
2. Are able to understand and comply with planned study procedures and be available for all study visits/phone calls.
3. Males or non-pregnant females ages 18-45, inclusive.
4. Subject must have a body mass index (BMI) $\geq$18.5 and <35 kg/m^2.
5. Are in good health*

* *As determined by medical history and physical examination to evaluate acute or currently ongoing chronic medical diagnoses or conditions, defined as those that have been present for at least 90 days which would affect the assessment of the safety of subjects or the immunogenicity of study vaccinations. Chronic medical diagnoses or conditions should be stable for the last 60 days (no hospitalizations, ER or urgent care for condition and no adverse symptoms that need medical intervention such as medication change/supplemental oxygen). This includes no change in chronic prescription medication, dose, or frequency as a result of deterioration of the chronic medical diagnosis or condition in the 60 days prior to enrollment. Any prescription change that is due to change of health care provider, insurance company, etc., or that is done for financial reasons, as long as in the same class of medication, will not be considered a deviation of this inclusion criterion. Any change in prescription medication due to improvement of a disease outcome, as determined by the site principal investigator or appropriate sub-investigator, will not be considered a deviation of this inclusion criterion. Subjects may be on chronic or as needed (prn) medications if, in the opinion of the site principal investigator or appropriate sub-investigator, they pose no additional risk to subject safety or assessment of reactogenicity and immunogenicity*

and do not indicate a worsening of medical diagnosis or condition. Similarly, medication changes subsequent to enrollment and study vaccination are acceptable provided there was no deterioration in the subject's chronic medical condition that necessitated a medication change, and there is no additional risk to the subject or interference with the evaluation of responses to study vaccination. Note: Topical, nasal, and inhaled medications (apart from steroids as outlined in the Subject Exclusion Criteria), herbals, vitamins, and supplements are permitted.

6. Oral temperature is less than 100.0 °F (37.8ºC).
7. Pulse is 47 to 105 beats per minute (bpm), inclusive.
8. Systolic blood pressure (BP) is 85 to 150 mm Hg, inclusive.
9. Diastolic blood pressure (BP) is 55 to 95 mm Hg, inclusive.
10. Have acceptable screening laboratories[#&] within 28 days prior to enrollment (Refer to Appendix C for acceptable screening values.)

[#] Screening labs include white blood cell (WBC), Hgb, platelet count, ANC, sodium, potassium, creatinine, albumin, total protein, PT, PTT, alanine aminotransferase (ALT). Blood Urea Nitrogen (BUN) will be obtained only if creatinine is above normal range.

[&] Screening laboratory values that are outside the range of eligibility but are thought to be due to an acute condition or due to laboratory error may be repeated once. [see Manual of Procedures (MOP)]

11. Have normal screening laboratories for urine protein. Trace protein is acceptable.
12. Drug screen for opiates is negative.
13. Hemoglobin A1C (HgbA1C) <6.3% at screening.
14. HIV 1/2 antibody negative.
15. HCV antibody negative.
16. HBsAg negative.
17. Women of childbearing potential[‡], must be using an effective method of contraception[§] from 30 days prior to the first study vaccination until 90 days after the second study vaccination.

[‡] *Not sterilized via tubal ligation, bilateral oophorectomy, hysterectomy,successful Essure® placement (permanent, non-surgical, non-hormonal sterilization) with history of documented radiological confirmation test at least 90 days after the procedure (or with use of another birth control method if history of confirmation test not confirmed), still menstruating, or < 1 year of the last menses if menopausal.*

[§] *Includes, but is not limited to, non-male sexual relationships, abstinence from sexual intercourse with a male partner, monogamous relationship with a vasectomized partner, male condoms with the use of applied*

spermicide, intrauterine devices, NuvaRing®, and licensed hormonal methods such as implants, injectables or oral contraceptives ("the pill").

18. Women of childbearing potential must have a negative serum pregnancy test at screening and a negative urine pregnancy test within 24 hours prior to each study vaccination.
19. Women agree to not donate eggs (ova, oocytes) from the start of screening onwards until at least 90 days after the second vaccination.
20. Agrees not to participate in another clinical trial during the study period.
21. Agrees not to donate blood to a blood bank for 3 months after receiving the second study vaccine.

5.2. Subject Exclusion Criteria

Subjects eligible to participate in this study must not meet any of the following exclusion criteria:

1. Women who are pregnant, planning to become pregnant or lactating*.

   * *Includes breastfeeding or planning to breastfeed at any given time from the receipt of study vaccination through the 6-month trial period.*

2. Known allergy or history of anaphylaxis, severe local or other serious adverse reactions to vaccines or vaccine products%, or history of severe allergic reactions.

   % *Includes a known allergy to egg, egg products and aminoglycosides or any of the constituents of the study vaccines [e.g., polysorbate 80, ethylenediaminetetraacetic acid (EDTA), L-histidine, tris (hydroxymethyl)-amino methane (THAM)).*

3. Received an experimental agent[5] within 3 months prior to Day 1, or expects to receive an experimental agent[6] during the 6-month trial-reporting period.

   [5] *Including vaccine, drug, biologic, device, blood product, or medication.*

   [6] *Other than from participation in this study.*

4. Received immunoglobulin or other blood product within 3 months before enrollment in this study.
5. Received any licensed live vaccine within 30 days prior to the first study vaccination through 30 days after the second study vaccination.
6. Received a licensed inactivated vaccine within 14 days prior to the first study vaccination through 14 days after the second study vaccination.
7. Has been vaccinated with an Ebola vaccine.

8. Has been diagnosed with Ebola disease, or exposed to Ebola virus including travel to West Africa^ in 2014-2016.

   ^ *West Africa includes but is not limited to the countries of Guinea, Liberia, Mali, Nigeria, and Sierra Leone.*

9. Known or suspected receipt of ChAd3-EBO-Z or other ChAd3-vectored vaccine.
10. Known or suspected receipt of an adenovirus serotype 5 (Ad5)-based vaccine.
11. Known or suspected receipt of any licensed or investigational small pox (vaccinia)-based vaccine[#].

    [#] *Includes any MVA-based candidate vaccine (Imvamune or Imvanex), Dryvax, or Acam2000.*

12. Has a typical vaccinia scar.
13. Confirmed Asplenia/Functional Asplenia.
14. A history of bleeding or clotting disorders.
15. Thyroidectomy or thyroid disease requiring medication during the last 12 months.
16. History of chronic urticaria (recurrent hives).
17. Individuals in whom the ability to observe possible local reactions at the eligible injection sites (left and right deltoid region) is, unacceptably obscured due to a physical condition or permanent body art.
18. Have an acute illness[#], as determined by the site PI or appropriate sub-investigator, within 72 hours prior to study vaccination.

    [#] *An acute illness which is nearly resolved with only minor residual symptoms remaining is allowable if, in the opinion of the site PI or appropriate sub-investigator, the residual symptoms will not interfere with the ability to assess safety parameters as required by the protocol. Subjects may re-screen after an acute illness is resolved.*

19. Any confirmed or suspected immunosuppressive or immunodeficient condition* or use of anticancer chemotherapy or radiation therapy (cytotoxic) within 3 years prior to study vaccination

    * *including HIV infection.*

20. Administration of chronic (defined as more than 14 days) immunosuppressants or other immune modifying drugs within 6 months of receipt of study vaccine.
21. Have taken oral or parenteral (including intraarticular) corticosteroids of any dose within 30 days prior to study vaccination.
22. Have taken high-dose[3] dose inhaled corticosteroids within 30 days prior to study vaccination.

[3] *High-dose defined using the inhaled high-dose reference chart (Appendix B).*

23. Have a history of convulsions or encephalomyelitis within 90 days prior to study vaccination.
24. Current or past history of alcohol or drug abuse in the last 5 years.
25. Subjects with autoimmune disorders, chronic inflammatory disorders or neurological disorders with a potential autoimmune correlation.
26. Have any diagnosis, current or past, of schizophrenia, bipolar disease, or other psychiatric diagnosis that may interfere with subject compliance or safety evaluations.
27. Have been hospitalized for psychiatric illness, history of suicide attempt, or confinement for danger to self or others within 10 years prior to study vaccination.
28. Have received any antiviral within 3 days of study vaccination
29. 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.*

30. Electrocardiogram (ECG) with clinically significant findings.*

    * *Clinically significant findings include the following:*

    a. *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 Bazett's formula [QTcB] [>450 ms]).*

    b. *Significant repolarization (ST-segment or T-wave) abnormality.*

    c. *Significant atrial or ventricular arrhythmia; frequent atrial or ventricular ectopy (e.g., frequent premature atrial contractions, 2 premature ventricular contractions in a row).*

    d. *ST-elevation consistent with ischemia or evidence of past or evolving myocardial infarction*

31. A diagnosis of Type I or II diabetes. (A history of isolated gestational diabetes is not an exclusion criterion).
32. Current employee or staff paid entirely or partially by the contract for this trial, or staff who are supervised by the PI or Sub-Investigators.
33. Any condition that would, in the opinion of the Site Investigator or appropriate sub-investigator, is a contraindication to study participation.*

    * *Including acute or chronic (persisting for at least 90 days) clinically significant medical disease or condition, that would place the subject at an unacceptable risk of injury, render the subject unable to meet the*

requirements of the protocol, or may interfere with the evaluation of responses or the subject's successful completion of the study.

5.3. Eligibility Criteria for Dose 2

Subjects may not receive subsequent vaccination if any of the criteria in Section 5.4.3 are met. The second vaccination may be deferred until eligibility criteria, as defined in Section 5.4.3, are met (see section 5.4.3).

5.4. Treatment Assignment Procedures

5.4.1. Randomization Procedures

Per International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline E6: Good Clinical Practice (GCP), screening records will be kept at the participating VTEU site to document the reason why an individual was screened, but failed trial entry criteria. The reasons why individuals failed screening will be recorded in the Statistical and Data Coordinating Center's AdvantageEDC^{SM} (Electronic Data Capture System).

Once consented and upon entry of demographic data and confirmation of eligibility for this trial, the subjects will be enrolled. Subjects will be randomized 1:1:1 in double-blind fashion to 1 dose of ChAd3-EBO-Z vaccine at Day 1 followed by a booster of placebo, ChAd3-EBO-Z (homologous), or MVA-BN®-Filo (heterologous) at Day 8.

Enrollment of subjects will be done online using the enrollment module of AdvantageEDC^{SM}. The randomization code will be prepared by statisticians at the SDCC and included in the enrollment module for this trial. AdvantageEDC^{SM} will assign each subject to a treatment arm after the demographic and eligibility data have been entered into the system. A designated individual at the participating VTEU site will be provided with a code list for emergency unblinding purposes, which will be kept in a secure place.

Instructions for use of the enrollment module are included in the AdvantageEDC^{SM} User's Guide. Manual back-up randomization procedures and instructions are provided for use in the event that a participating VTEU site temporarily loses access to the Internet or the online enrollment system is unavailable.

Subjects who sign the informed consent form (ICF) and are randomized but do not receive study vaccine may be replaced. Subjects who sign the informed consent form, and are randomized and vaccinated, and subsequently withdraw, or are withdrawn or terminated from this trial, or are lost to follow-up may be replaced.

5.4.2. Masking Procedures

This is a double-blind clinical trial. Subjects, investigators, study personnel performing any study-related assessments following study vaccine administration, and laboratory personnel performing antibody assays will be blinded to treatment arm assignment (i.e., type of booster). Laboratory personnel performing experimental assays including antibody assays will also be masked to subject ID and study visit.

The randomization scheme will be generated by the SDCC and provided to unblinded study personnel (i.e., research pharmacists performing study vaccination preparations and unblinded study vaccine administrators) at the participating VTEU site. To preserve blinding and mask the color of study product in the syringe, the unblinded site research pharmacist will place a blinding tape or overlay on the syringe to mask its content. The vaccine will then be sent to an unblinded study vaccine administrator for administration to the subject.

The unblinded study vaccine administrator is a study personnel member credentialed to administer vaccines and may also participate in dose preparation, but will not be involved in study-related assessments or have subject contact for data collection following study vaccine administration.

The Safety Monitoring Committee (SMC) may receive data in aggregate and presented by treatment arm. The SMC may also be provided with expected and observed rates of the expected AEs in an unblinded fashion, and may request the treatment arm be unblinded for an individual subject if required for safety assessment. The SMC will review grouped and unblinded data in the closed session only. Refer to the protocol-specific Manual of Procedures (MOP) for unblinding procedures.

5.4.3. Discontinuation of Study Product

There are several reasons why a study subject may be prevented from receiving the second vaccination. 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.

The second study vaccination will not be administered to a subject if any of the following criteria are met:

- Pregnancy
- Receipt of disallowed licensed vaccine, experimental product or medication (see Section 5.2)
- New onset of illness or condition that meets the Exclusion Criteria (see Section 5.2)
- Medical condition or medication change for which continued participation, in the opinion of the site principal investigator or appropriate sub-investigator, would pose a risk to the subject or would likely confound interpretation of the results. Note: Medication changes subsequent

to the first study vaccination are not exclusionary for receipt of the follow-up study vaccination provided there was no deterioration in the subject's chronic medical condition that necessitated a medication change, and there is no additional risk to the subject or interference with the evaluation of responses to study vaccination.

- Presence of signs or symptoms that could confound or confuse assessment of study vaccine reactogenicity.

Note: for subjects with transient injection site or systemic signs or symptoms, or with an acute illness, including an oral temperature greater than or equal to 100.0°F, follow-up study vaccination/s should be postponed/deferred until signs, symptoms, or acute illness have resolved, or are improving as further specified below. No study vaccine will be given outside the protocol-specified window.

Note for afebrile, acute illness only: If a subject is afebrile, his/her acute illness is nearly resolved with only minor residual symptoms remaining, this occurs within the acceptable protocol-specified window for that visit, and, in the opinion of the site principal investigator or appropriate sub-investigator, the residual symptoms will not interfere with the ability to assess safety parameters as required by the protocol, the subject may receive the follow-up study vaccination without DMID Medical Officer approval.

- Grade 3 solicited or unsolicited adverse event that is ongoing, whether or not it is improved or resolving. An unresolved or continuing Grade 1 or Grade 2 adverse event is permissible following the documented determination by the site principal investigator or appropriate sub-investigator, that it would not render study vaccination unsafe or interfere with the evaluation of adverse events or immunologic response.
- Grade 3 solicited or unsolicited adverse event that occurs in the 7 days following study vaccination, lasts for more than 24 hours without decreasing to a Grade 1 or Grade 2, and does not have an alternative etiology.
- Any laryngospasm, bronchospasm, or anaphylaxis within 1 day after administration of study product that is considered related to study product.
- Any generalized urticaria within 3 days after administration of study product that is considered related to study product.
- Serious adverse event related to the study vaccination.
- As deemed necessary by the site principal investigator or appropriate sub-investigator for noncompliance or other reasons.
- Subject refusal of further study vaccination.

- Termination of this trial.
- New information becomes available that makes further administration of the study vaccine unsafe.

Subjects who receive the first study vaccine but not the second vaccination will be encouraged to remain in this trial for follow-up, safety and immunogenicity assessments per Appendix A: Schedule of Study Procedures and Evaluations. If the scheduled visit does not include collection of blood for safety or immunogenicity, the visit may be conducted by phone call/ electronic communication (e.g., email, text message) rather than in person continuing through approximately 6 months after the first study vaccination. See the protocol-specific Manual of Procedures (MOP) for alternate follow-up requirements.

Every attempt will be made to follow all adverse events, including solicited local site and systemic AEs, unsolicited non-serious adverse events, SAEs and MAAEs ongoing at the time of early withdrawal through resolution as per applicable collection times defined for the specific type of adverse event.

5.4.4. Study Withdrawal

Subjects may voluntarily withdraw their consent for trial participation at any time and for any reason, without penalty. The site principal investigator or appropriate sub-investigator may also choose to remove a subject from the study. A subject may withdraw or be withdrawn from this trial for any of the following reasons:

- Medical disease or condition, or any new clinical finding for which continued participation, in the opinion of the site principal investigator or appropriate sub-investigator, would compromise the safety of the subject, or would interfere with the subject's successful completion of this trial, or would interfere with the evaluation of adverse events or immunologic response.
- As deemed necessary by the site principal investigator or appropriate sub-investigator for noncompliance or other reasons.
- Subject withdrawal of consent.
- Subject lost to follow-up.
- Subject dies.
- Termination of this trial.
- New information becomes available that makes further participation unsafe.

In the case of subjects who fail to appear for a follow-up safety assessment, extensive effort (i.e., three documented contact attempts via phone calls, emails, text messages, etc., made on separate occasions and followed by a certified letter) will be made to locate or recall them, or at least to determine their health status. These efforts will be documented in the subject's study records.

5.4.5. Handling of Withdrawals

The primary reason for withdrawal from this trial will be recorded on the Study Status data collection form. Subjects will be encouraged to complete the Early Termination Visit. The Early Termination Visit procedures are listed in Section 7.5.

Subjects who withdraw, or are withdrawn or terminated from this trial, or are lost to follow-up after signing the ICF, randomization, and receipt of study vaccine may be replaced to ensure each vaccine arm has at least 20 evaluable subjects, up to a total of 60 evaluable subjects. In order to be evaluable, a subject must receive the ChAd3-EBO-Z vaccine at Visit 1 and the booster vaccination of either ChAd3-EBO-Z, MVA-BN®-Filo, or placebo on Day 8 as determined by randomization.

Subjects who withdraw, or are withdrawn or terminated from this trial, or are lost to follow-up after signing the ICF and randomization but before receipt of study vaccine may be replaced.

5.4.6. Termination of Study

Although the sponsor has every intention of completing this trial, it reserves the right to terminate this trial at any time for clinical or administrative reasons. Reasons for termination include, but are not limited to, study closure due to the recommendation after a SMC review and at the discretion of DMID.

If the study is prematurely terminated by the sponsor, any regulatory authority, or the investigator for any reason, the investigator will promptly inform the study subjects and assure appropriate therapy or follow-up for the subjects, as necessary. The investigator will provide a detailed written explanation of the termination to the IRB/IEC.

6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1. Study Product Description

The ChAd3-EBO-Z vaccine consists of a recombinant replication-defective chimpanzee adenovirus Type 3 vector engineered to express the WT GP antigen from EBOV. The vaccine is formulated in a buffer (without addition of a preservative). The formulation buffer, pH 7.4, is composed of 10 mM Tris, 10 mM Histidine, 5% Sucrose (w/v), 75 mM Sodium Chloride, 1 mM Magnesium Chloride, 0.02% Polysorbate 80 (PS-80) (w/v), 0.1 mM EDTA, and 0.5% Ethanol (v/v).

The MVA-BN®-Filo vaccine is based on the highly attenuated virus vector, Modified Vaccinia Ankara Bavarian Nordic (IMVAMUNE®). The recombinant vaccine encodes the glycoproteins of Ebola Virus Sudan, Ebola Virus Zaire, and Marburg Virus Musoke and the nucleoprotein of Tai Forest Ebola virus. The vaccine is manufactured in Chicken Embryo Fibroblast (CEF) cells derived from Specific Pathogen Free (SPF) eggs in a suspension culture using serum free medium.

6.1.1. Acquisition

ChAd3-EBO-Z

ChAd3-EBO-Z will be provided by GlaxoSmithKline.

MVA-BN®-Filo

MVA-BN®-Filo will be provided by Janssen Vaccines and Prevention BV in collaboration with Bavarian Nordic.

Placebo

Placebo, 0.9% Sodium Chloride Injection, USP will be provided by the DMID Clinical Materials Services, Fisher BioServices.

Upon request by DMID, MVA-BN®-Filo and ChAd3-EBO-Z will be shipped to the following address:

DMID Clinical Materials Services
Fisher BioServices
20439 Seneca Meadows Parkway
Germantown, MD 20876
Phone: 240-477-1350
Fax: 240-477-1360
Email: DMID.CMS@ThermoFisher.com

MVA-BN®-Filo, ChAd3-EBO-Z and placebo will be provided through the DMID Clinical Materials Services to the participating VTEU site prior to the start of this trial upon request and with prior approval from DMID. Should the site principal investigator require additional vaccine during this trial, further instructions are provided in the protocol-specific MOP.

6.1.2. Formulation, Packaging, and Labeling

ChAd3-EBO-Z

ChAd3-EBO-Z is presented as a clear, sterile liquid suspension filled in stoppered glass vials. The nominal titer for the vaccine is 2×10^{11} vp/mL. Vials have a fill volume of approximately 1.2 mL. 1.0 mL is to be administered IM.

The study product will be labeled according to manufacturer specifications and include the statement "Caution: New Drug – Limited by Federal Law to Investigational Use".

MVA-BN®-Filo

MVA-BN®-Filo is a milky, light yellow suspension with no visible extraneous particles and contains trometamol (tris-hydroxymethyl-amino methane) and sodium chloride as formulation buffers.

The vaccine is supplied in a 2mL type I borosilicate glass vial closed with a sterile bromobutyl rubber stopper, crimped with an aluminum cap and covered with a polypropylene closure. The nominal titer for the vaccine is 2.0×10^{8} Inf. U/mL. Vials are filled with an extra amount of vaccine (fill volume: 0.69 mL) to assure an extractable volume of 0.5mL.

The study product will be labeled according to manufacturer specifications and include the statement "Caution: New Drug – Limited by Federal Law to Investigational Use".

Placebo

Placebo will be supplied as 0.9% Sodium Chloride Injection, USP which is a colorless, sterile, nonpyrogenic, isotonic solution of sodium chloride and water for injection (WFI). Each mL contains sodium chloride 9 mg. It contains no bacteriostatic, antimicrobial agent, or added buffer and is supplied only in single-dose containers. The placebo, 0.9% Sodium Chloride, contains no preservatives. The solution may contain hydrochloric acid and/or sodium hydroxide for pH adjustment (pH 5.3 [4.5 to 7.0]). 0.5 mL is to be administered IM.

6.1.3. Product Storage and Stability

ChAd3-EBO-Z

ChAd3-EBO-Z vaccine vials must be stored at -60°C to -90°C. If the vaccine cannot be administered immediately, it is recommended to administer the product within 6 hours after thawing.

MVA-BN®-Filo

MVA-BN®-Filo vaccine vials must be stored at -20°C to -80°C. If the vaccine cannot be administered immediately it is recommended to administer the product within 4 hours after preparation in syringe.

Placebo

Placebo must be stored at 20ºC to 25ºC (68°F to 77ºF) [See USP Controlled Room Temperature; excursions between 15°C and 30°C (59°F and 86°F) are permitted]. See protocol-specific MOP for further instructions.

The temperature of the storage unit must be recorded manually daily (excluding non-business days and holidays as applicable), continuously monitored and recorded during the duration of this trial per the participating VTEU site's standard operating procedures (SOPs), and documentation will be maintained. If the temperature fluctuates outside of the required range, the affected study product(s) must be quarantined at the correct storage temperature and labeled as 'Do Not Use' (until further notice). The research pharmacist must alert the site principal investigator and study coordinator, if the temperature fluctuates outside of the required range. In the event the temperature fluctuates outside of the required range, the affected study product(s) must not be administered. The site principal investigator or responsible person should immediately contact the DMID Product Support Team at DMIDProductSupportTeam@niaid.nih.gov for further instructions before any additional study vaccinations are administered. Based on the information collected, DMID and/or the manufacturer will determine whether the affected study product(s) can be used. If it cannot be used, the site will receive specific instructions on how to return the affected study product(s) to the DMID CMS or destroy it on site. Additional instructions for quarantine are provided in the protocol-specific MOP.

6.2. Dosage, Preparation and Administration of Study Intervention/ Investigational Product

See the protocol-specific MOP for detailed information on the preparation, labeling, storage, and administration of study product for each treatment arm. Study product preparation will be performed by the participating VTEU site's research pharmacist on the same day of study vaccine administration.

Visually inspect the study product upon receipt and prior to use. If the study product(s) appear(s) to have been damaged, contaminated or discolored, contain visible particulate matter or if there

are any concerns regarding its integrity, do NOT use the affected study product(s). The affected study product(s) must be quarantined as per storage requirements and labeled as 'Do Not Use' (until further notice). The site principal investigator or responsible person should immediately contact the DMID Product Support Team at DMIDProductSupportTeam@niaid.nih.gov and DMID Clinical Project Manager for further instructions before any additional study vaccinations are administered. Based on the information collected, DMID and/or the manufacturer will determine whether the affected study product(s) can be used. If it cannot be used, the site will receive specific instructions on how to return the affected study product(s) to the DMID CMS or destroy it on site. If the study product is unusable, study personnel will use another vial from the study supply. Replacement vials may be requested by contacting DMID. Additional instructions for quarantine and DMID contact information are provided in the protocol-specific MOP.

ChAd3-EBO-Z

The study product will be prepared for administration by thawing at room temperature. Once the last ice crystals have disappeared within the thawed vials, the vial should be gently shaken to homogenize the suspension. After thawing, the drug product should appear as transparent and without precipitates. The vaccine should be visually inspected for any foreign particulate matter prior to administration. In case foreign particulate matter is visable, the vaccine must not be used. The final dose of vaccine will be 2×10^{11} vp.

MVA-BN®-Filo

The study product will be prepared for administration by thawing at room temperature. To ensure homogeneity, upon thawing the vial should be swirled gently (not shaken) for at least 30 seconds. After thawing, the drug product should appear as a clear to milky colored suspension. The liquid vaccine should be visually inspected for any foreign particulate matter prior to administration. In case foreign particulate matter is visible, the vaccine must not be used. The final dose of vaccine will be 1×10^{8} IU.

The vaccines will be administered via intramuscular injection into the deltoid. For the second injection, subjects will receive study vaccine or placebo via intramuscular injection into the opposite arm that was used for the first injection (left or right deltoid). Study product administration will be performed by an unblinded study personnel member who is credentialed to administer vaccines and may also participate in dose preparation, but will not be involved in study-related assessments or have subject contact for data collection following study vaccine administration.

6.3. Modification of Study Intervention/Investigational Product for a Subject

There will be no dose modifications. If a subject's second vaccination is deferred, attempts will be made to reschedule the vaccination to occur within the acceptable protocol-specified window for that visit.

6.4. Accountability Procedures for the Study Intervention/Investigational Product(s)

After receipt of the study vaccine, the site principal investigator is responsible for its distribution and disposition, and has ultimate responsibility for study vaccine accountability. As this is a blinded study, the site PI will delegate this responsibility to the unblinded site pharmacist. Study vaccine records must be maintained and document logs of receipt, accountability, and storage temperature conditions. The study product accountability records and dispensing logs will also capture vial numbers, including final vial number, date of study vaccine preparation/ administration, time of study vaccine preparation, time study vaccine is drawn into the syringe, and amount of study vaccine withdrawn for administration. Time of study vaccine administration to the subject will be captured on the appropriate data collection form. All study vaccine, whether administered or not, must be documented on the appropriate study product accountability record or dispensing log. The sponsor's monitoring staff will verify the participating VTEU site's study product accountability records and dispensing logs per the site monitoring plan.

Used and unused vaccines, will be retained until monitored and released for disposition as applicable. Filled, un-used syringes will be retained until monitored and released for disposition as applicable. Final disposition of the unused vaccine will be determined by DMID and communicated to the participating VTEU site by the DMID Clinical Project Manager.

6.5. Assessment of Subject Compliance with Investigational Product

Study products will be administered to subjects via intramuscular injection by study personnel according to subject treatment assignment and as described in Section 6.2 Thus, subject compliance is not anticipated to be an issue. Deviations from the dose schedule may only occur as described in Section 6.3.

6.6. Concomitant Medications/Treatments

Administration of any medications, therapies, or vaccines will be recorded on the appropriate data collection form. Concomitant medications will include all current medications and medications taken within 60 days prior to signing the informed consent form through approximately 28 days after the second study vaccination or early termination (if prior to 28 days

after the second study vaccination), whichever occurs first. Assessment of study eligibility will include a review of all permitted and prohibited medications per the Subject Inclusion and Exclusion Criteria (see Section 5). Medications reported in the electronic case report form (eCRF) are limited to those taken within 30 days prior to study vaccination through approximately 28 days after the second study vaccination. Prescription and over-the-counter drugs will be included as well as herbals, vitamins, and supplements. In addition, receipt of non-study vaccines will be solicited through approximately 28 days after the second study vaccination, and reported in the eCRF.

Use of new medication should prompt evaluation for the occurrence of an AE or worsening of a pre-existing medical condition.

Medications that might interfere with the evaluation of the investigational product(s) should not be used from time of study vaccination through 28 days post the second vaccination unless clinically indicated as part of the subject's health care. Medications in this category include the prohibited medications per the Subject Exclusion Criteria (see Section 5.2). In addition, the site principal investigator or appropriate sub-investigator may identify other medications that should not be used due to a risk to subject safety or assessment of reactogenicity and immunogenicity. Use of medications as prophylaxis prior to study vaccination is prohibited. There are no known drug-vaccine interactions with the study vaccine and subjects are not being asked to discontinue current medications not listed in the exclusion criteria. In the event medical conditions dictate use of medications, subjects are encouraged to obtain adequate care, comply with the course of therapy as prescribed by their physician and inform the site as soon as practicable. Details of all medications taken during the medication reporting period for this study (date, indication, brand or generic name) will be recorded.

7 STUDY SCHEDULE

Complete study schedule details listed by type of visit are described below. Refer also to Sections 4 and 8, and Appendix A: Schedule of Study Procedures and Evaluations.

7.1. Screening

Day < - 29 to -1, Visit 00

Potential subjects will be screened for eligibility within 28 days prior to the administration of study vaccination. The following activities will be performed at screening and may be done all at one visit or split into separate visits:

- Subjects will be provided with a description of this trial (purpose and study procedures) and asked to read and sign the informed consent form. The informed consent form will be signed prior to performing any study procedures, including any screening procedures.
- Demographic information will be obtained by interview of subjects.
- History of military service prior to 1991 or after January 2003. Subjects will be interviewed for possible receipt of vaccinia-based vaccine.
- Eligibility criteria will be reviewed with subjects.
- Complete medical history will be obtained by interview of subjects.
- History of all concomitant medications taken within 60 days prior to signing the informed consent form will be reviewed with subjects. Medications reported in the eCRF are limited to those taken within 30 days prior to study vaccination. Assessment of study eligibility will include a review of all permitted and prohibited medications per the Subject Inclusion and Exclusion Criteria (see Section 5).
- Vital signs, including oral temperature, pulse, and blood pressure, will be obtained. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- Height and weight will be collected. BMI will be calculated to determine eligibility.
- An ECG will be performed and read to determine eligibility.
- A physical examination will be performed to include the following organs and organ systems: general appearance, skin, head and neck, lungs, heart, liver, spleen, extremities, musculoskeletal, lymph nodes, and nervous system by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

- A serum pregnancy test will be performed on all females of childbearing potential and must be negative.
- Venous blood will be collected for screening laboratory tests (refer to Section 8.2.1) to be run at the local clinical laboratory.
- Urine will be collected for screening urinalysis (dipstick) for urine protein and urine toxicology screen for opiates. Values must meet the eligibility criteria (see Section 5) prior to randomization.

7.2. Enrollment/Baseline

Day 1, Visit 1

Sixty subjects who meet all inclusion and no exclusion criteria will be administered study product. The following procedures will occur for all Treatment Arms:

- Subject's willingness to participate will be reconfirmed and documented in the subject's study records prior to performing any further study procedures, including administration of the study vaccine.
- Eligibility criteria, including results of all clinical screening laboratory evaluations, will be reviewed with subjects prior to study vaccination to ensure continued eligibility.
- Complete medical history and any updates obtained by interview of subjects since the screening visit will be reviewed with subjects prior to study vaccination to ensure continued eligibility.
- All concomitant medications will be reviewed with subjects prior to study vaccination for accuracy and completeness. Any new concomitant medications (including non-study vaccines) taken since the screening visit will be reviewed with subjects and assessed for continued eligibility prior to study vaccination. Medications reported in the eCRF are limited to those taken within 30 days prior to study vaccination.
- Vital signs, including oral temperature, pulse, and blood pressure, will be obtained to ensure eligibility. Vital signs assessed on Day 1 prior to the first study vaccination will be considered as baseline. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- A targeted physical examination may be performed prior to study vaccination, if indicated based on review of complete medical history and any updates obtained by interview of subjects since the screening visit, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

- A urine pregnancy test will be performed within 24 hours prior to study vaccination on all females of childbearing potential. Results must be negative and known prior to randomization and study vaccination.
- Subjects will be enrolled in AdvantageEDC^{SM} and assigned randomly to a treatment arm prior to study vaccination.
- Venous blood will be collected prior to the study vaccination for safety labs (refer to Section 8.2.1).
- Venous blood will be collected prior to the study vaccination for high resolution HLA typing for Class 1 (A, B, and C locus) testing.
- Venous blood will be collected prior to the study vaccination for future use assays.
- Venous blood will be collected prior to the study vaccination for baseline immunogenicity and exploratory assays (please refer to Appendix A: Schedule of Study Procedures and Evaluations for type of assays requiring collection).
- Pre-administration reactogenicity assessments will be performed prior to study vaccination to establish a baseline. Subjects will then receive study vaccine or placebo via intramuscular injection into the left or right deltoid. The time of administration will be recorded on the appropriate data collection form. Subjects will be observed for at least 30 minutes after study vaccination. Post-administration reactogenicity assessments will be performed. Any AE/SAEs/MAAEs will be recorded on the appropriate data collection form prior to discharge from the clinic.
- Subjects will be provided with a memory aid and other study-related materials to record daily oral temperature, solicited site and systemic AEs, unsolicited AEs, and concomitant medications. Subjects will be encouraged to take their oral temperature around the same time each day. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature. Subjects will be instructed on how to use their memory aid and how to measure and record AEs prior to discharge from the clinic. Subjects will be instructed to notify the study center if they develop any severe reactions after study vaccination. If the site principal investigator or appropriate sub-investigator deems the reaction severe enough, further instructions will be given to the subject on the proper course of action, including a return to the clinic for immediate evaluation if appropriate.

7.3. Follow-up

7.3.1. Follow-up Visits, post first vaccination

Day 2 (V02), 3 (V03), 4 (V04) and 6 (V05)

Follow-up visits are scheduled in reference to study vaccination date as indicated for each visit window. All subjects will return on D2, 3, 4, 6 post the first vaccination. The following assessments will occur:

- Interim medical history will be obtained by interview of subjects to ensure continued eligibility. Any changes since the previous clinic visit or contact will be noted at each clinic visit.
- All concomitant medications (including solicitation for receipt of any non-study vaccines) will be recorded on the appropriate data collection form at each clinic visit. Any new concomitant medications taken since the last clinic visit or phone contact will be reviewed with subjects and assessed for continued eligibility.
- A targeted physical examination may be performed, if indicated based on review of complete medical history and any updates obtained by interview of subjects, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Venous blood will be collected for immunogenicity and exploratory assays (please refer to Appendix A: Schedule of Study Procedures and Evaluations for type of assays requiring collection).
- All AEs, SAEs and MAAEs will be collected and recorded on the appropriate data collection form at each clinic visit.
- Memory aid information will be reviewed with subjects at each clinic visit.

7.3.2. Follow-up Vaccination Visit

Day 8 +2 (post first vaccination), Visit 06

All subjects will return for vaccination on D8. The following assessments will occur.

- Eligibility criteria will be reviewed with subjects prior to study vaccination to ensure continued eligibility (see Section 5.3).
- Interim medical history will be obtained by interview of subjects to ensure continued eligibility. Any changes since the previous clinic visit or contact will be noted at each clinic visit.
- All concomitant medications (including solicitation for receipt of any non-study vaccines) will be recorded on the appropriate data collection form at each clinic visit. Any new concomitant medications taken since the last clinic visit or phone contact will be reviewed with subjects and assessed for continued eligibility.

- Vital signs, including oral temperature, pulse, and blood pressure, will be obtained to ensure continued eligibility. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- A targeted physical examination may be performed prior to study vaccination, if indicated based on review of complete medical history and any updates obtained by interview of subjects since the screening visit, by a study clinician listed on the Form FDA 1572.
- A urine pregnancy test will be performed within 24 hours prior to study vaccination on all females of childbearing potential. Results must be negative and known prior to randomization and study vaccination.
- Venous blood will be collected prior to the study vaccination for safety labs (See Section 8.2.1).
- Venous blood will be collected prior to the study vaccination for future use assays.
- Venous blood will be collected prior to the study vaccination for immunogenicity and exploratory assays (please refer to Appendix A: Schedule of Study Procedures and Evaluations for type of assays requiring collection).
- Pre-administration reactogenicity assessments will be performed prior to study vaccination to establish a baseline. Subjects will then receive study vaccine or placebo via intramuscular injection into the opposite arm that was used for the first injection (left or right deltoid). The time of administration will be recorded on the appropriate data collection form. Subjects will be observed for at least 30 minutes after study vaccination. Post-administration reactogenicity assessments will be performed. Any AEs, SAEs, and MAAEs will be recorded on the appropriate data collection form prior to discharge from the clinic.
- Subjects will be provided with a memory aid and other study-related materials to record daily oral temperature, solicited site and systemic AEs, unsolicited AEs, and concomitant medications. Subjects will be encouraged to take their oral temperature around the same time each day. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature. Subjects will be instructed on how to use their memory aid and how to measure and record AEs prior to discharge from the clinic. Subjects will be instructed to notify the study center if they develop any severe reactions after study vaccination. If the site principal investigator or appropriate sub-investigator deems the reaction severe enough, further instructions will be given to the subject on the proper course of action, including a return to the clinic for immediate evaluation if appropriate.
- All AEs, SAEs, and MAAEs will be collected and recorded on the appropriate data collection form at each clinic visit.

7.3.3. Follow-up Visits, post second vaccination

Day 2 (V07), 8 +1 (V08), 15 +2/-1 (V09), 22 +/-1 (V10), 29 +/-2 (V11) post second vaccination

Follow-up visits are scheduled in reference to study vaccination date as indicated for each visit window. All subjects will return on D2, 8, 15, 22 and 29 post the second vaccination. The following assessments will occur:

- Interim medical history will be obtained by interview of subjects to ensure continued eligibility. Any changes since the previous clinic visit or contact will be noted at each clinic visit.
- All concomitant medications (including solicitation for receipt of any non-study vaccines) will be recorded on the appropriate data collection form at each clinic visit. Any new concomitant medications taken since the last clinic visit or phone contact will be reviewed with subjects and assessed for continued eligibility.
- A targeted physical examination may be performed, if indicated based on review of complete medical history and any updates obtained by interview of subjects, by a study clinician listed on the Form FDA.
- Venous blood will be collected for immunogenicity and exploratory assays (please refer to Appendix A – Schedule of Study Procedures and Evaluations for type of assays requiring collection).
- Venous blood will be collected for safety labs only on Day 8 (+1), 22 (+/-1), and 29 (+/-2) post second vaccination (See Section 8.2.1).
- Venous blood will be collected for future use assays on Day 8 (+1), 15 (+2/-1), and 29 (+/-2) post second vaccination (See Section 8.2.1).
- All AEs, SAEs, and MAAEs will be collected and recorded on the appropriate data collection form at each clinic visit.
- Memory aid information will be reviewed with subjects on Day 2 and 8 post second vaccination.

7.3.4. Follow-up Phone Calls

Day 76 +/-7 (V12), Day 106 +/-7 (V13) post first vaccination

Follow-up visits are scheduled in reference to study vaccination date as indicated for each visit window. All subjects will be called at approximately D76 and 106 post the first vaccination. The following assessments will occur:

- Interim medical history will be reviewed to ensure continued eligibility. Any changes since the previous clinic visit or contact will be noted at the phone call.
- All SAEs and MAAEs will be collected and recorded on the appropriate data collection form at the phone call.

7.4. Final Study Visit

Day 182 +/-14 post first vaccination, Visit 14

Final visits are scheduled in reference to study vaccination date as indicated for each visit window. All subjects will return on D182 post the first vaccination for a final study visit. The following assessments will occur:

- Interim medical history will be obtained by interview of subjects. Any changes since the previous clinic visit or contact will be noted.
- A targeted physical examination may be performed, if indicated based on review of complete medical history and any updates obtained by interview of subjects, by a study clinician listed on the Form FDA 1572.
- Venous blood will be collected for future use assays.
- All SAEs and MAAEs will be collected and recorded on the appropriate data collection form.

7.5. Early Termination Visit (if needed)

The following activities will be performed on all subjects at the early termination visit on subjects who withdraw, or are withdrawn or terminated from this trial:

- Interim medical history will be obtained by interview of subjects and any changes since the previous clinic visit or contact will be noted (if indicated).
- Memory aid information will be reviewed with subjects (if within 7 days post study vaccination).
- All concomitant medications (including solicitation for receipt of any non-study vaccines) will be recorded on the appropriate data collection form (if prior to 28 days post second study vaccination).
- All AE, SAEs, and MAAEs will be recorded on the appropriate data collection form. AEs will be recorded if prior to 28 days post second study vaccination. AEs will be limited to SAEs and MAAEs, if after 28 days post first study vaccination.
- A targeted physical examination may be performed, if indicated based on review of interim medical history, by a study clinician licensed listed on the Form FDA 1572.

- Vital signs, including oral temperature, pulse, and blood pressure maybe obtained.Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- Venous blood will be collected for safety laboratory evaluations (see Section 8.2.1) and performed by the local (clinical) laboratory (if within 28 days post second study vaccination).
- Venous blood will be collected for immunogenicity and exploratory assays.

7.6. Unscheduled Visit (if needed)

Unscheduled visits may occur at any time during this trial. Any of the following activities may be performed:

- Interim medical history will be obtained by interview of subjects and any changes since the previous clinic visit or contact will be noted (if indicated).
- Memory aid information will be reviewed with subjects (if within 7 days post study vaccination).
- All concomitant medications (including solicitation for receipt of any non-study vaccines) will be recorded on the appropriate data collection form (if prior to 28 days post second study vaccination).
- All AE, SAEs, and MAAEs will be recorded on the appropriate data collection form. AEs will be recorded if prior to 28 days post last study vaccination. AEs will be limited to SAEs and AEs if after 28 days post first study vaccination.
- A targeted physical examination may be performed, if indicated based on review of interim medical history, by a study clinician licensed listed on the Form FDA 1572.
- Vital signs, including oral temperature, pulse, and blood pressure, may be obtained. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- Venous blood will be collected for safety laboratory evaluations (see Section 8.2.1) and performed by the local (clinical) laboratory (if within 28 days post second study vaccination).

8 STUDY PROCEDURES/EVALUATIONS

8.1. Clinical Evaluations

Complete medical history will be obtained by interview of subjects at the screening visit and will be reviewed and/or updated on Day 1 prior to study vaccination. Subjects will be queried regarding a history of significant medical disorders of the head, eyes, ears, nose, throat, mouth, cardiovascular system, lungs, gastrointestinal tract, liver, pancreas, kidney, urologic system, nervous system, blood, lymph nodes, endocrine system, musculoskeletal system, skin, and genital/reproductive tract. A history of any allergies, cancer, immunodeficiency, psychiatric illness, substance abuse, and autoimmune disease will be solicited. At follow-up visits after study vaccination, an interim medical history will be obtained by interview of subjects noting any changes since the previous clinic visit or contact. The interim medical history should include an assessment for new medical conditions.

Medication history (concomitant medications) will include a review of all current medications and medications taken within 60 days prior to signing the informed consent form through approximately 28 days after the second study vaccination or early termination (if prior to 28 days after the second study vaccination), whichever occurs first. Medications reported in the eCRF are limited to those taken within 30 days prior to study vaccination through approximately 28 days after the second study vaccination. Prescription and over-the-counter drugs will be included as well as herbals, vitamins, and supplements. In addition, receipt of non-study vaccines will be solicited through approximately 28 days after the second study vaccination, and reported in the eCRF. Use of new medication should prompt evaluation for the occurrence of any AE. Assessment of eligibility will include a review of all permitted and prohibited medications per the Subject Inclusion and Exclusion Criteria (see Sections 5.1 and 5.2). In addition, the site principal investigator or appropriate sub-investigator may identify other medications that should not be used due to a risk to subject safety or assessment of reactogenicity and immunogenicity.

A full physical examination will be performed on all subjects at the screening visit. This exam will include the following organs and organ systems: skin, head and neck, lungs, heart, liver, spleen, extremities, musculoskeletal, lymph nodes and nervous system, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. Vital signs (oral temperature, pulse, and blood pressure) will be collected at the screening visit. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature. Height and weight will be collected at the screening visit. BMI will be calculated, and eligibility determined.

A single, 12-lead ECG will be performed at screening and will be read locally. Electrocardiograms will only be repeated during the study if clinically indicated based on signs and symptoms (per the investigator's judgment). During the collection of ECGs, subjects should

be in supine position in a quiet setting without distractions. Subjects should rest (while sitting comfortably) for at least 5 minutes before ECG collection and should refrain from talking or moving arms or legs.

On Day 1 prior to the study vaccination and at follow-up visits after the study vaccination, a targeted physical examination may be performed, if indicated based on the subject's interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

On Day 1 and all vaccination visits, vital signs (oral temperature, pulse, and blood pressure) will be collected prior to study vaccination. A urine pregnancy test will be performed within 24 hours prior to study vaccination on all females of childbearing potential. Pre-administration reactogenicity assessments will be performed prior to each study vaccination to establish baseline, then the study vaccination will be given. Subjects will be observed in the clinic for at least 30 minutes after each study vaccination. The study vaccination site will be examined, post-administration reactogenicity assessments will be performed and any AE, SAEs and MAAEs will be recorded on the appropriate data collection form prior to discharge from the clinic. All subjects will receive and complete a subject memory aid from the time of study vaccination through 7 days after study vaccination. Subject memory aids will be reviewed with the subjects for adverse events, (solicited local and systemic AEs and unsolicited AEs) approximately at day 8 after each vaccination.

Reactogenicity assessments will include an assessment of solicited adverse events occurring from the time of study vaccination through 7 days after study vaccination. These include local reactions such as pain, tenderness, erythema (redness), induration (hardness/swelling), pruritic (itching) and bruising as well as systemic AEs such as fever, feverishness (chills, shivering, sweating), fatigue (tiredness), malaise (general unwell feeling), myalgia (body aches/muscular pain), nausea, loss of appetite and headache.

8.2. Laboratory Evaluations

8.2.1. Clinical Laboratory Evaluations

Serum βHCG pregnancy tests will be done at the screening visit for all females of childbearing potential and processed locally by the site laboratory. A urine pregnancy test will be performed within 24 hours prior to each study vaccination on all females of childbearing potential. Urine will be collected in a sterile urine cup for processing by research staff using a CLIA waived urine pregnancy test kit. Results must be negative and known prior to randomization on Day 1 and prior to administration of study vaccination on Day 8.

Venous blood will be collected prior to the first study vaccination on day 1 for high resolution HLA typing for Class 1 (A, B, and C locus) testing.

Clinical screening laboratory tests will be performed at the screening visit and processed by the local (clinical) laboratory. Parameters to be evaluated to confirm trial eligibility as per Section 5.1, include the following:

- Hematology: WBC, Hgb, platelet count, ANC, and HgbA1c.
- Biochemistry: Creatinine, sodium, potassium, albumin, total protein and ALT. BUN will be done if creatinine is above the normal range.
- Serum serology testing for HIV – 1/2 antibody, HCV antibody, and HBsAg.
- Clotting: PT and PTT
- Urinalysis will be done to test for urine protein via dipstick in the clinic. Clean-catch, mid-stream, urine specimen will be collected in a sterile urine cup.
- Urine test for opiates: A clean-catch, mid-stream urine specimen will be collected in a sterile urine cup and transported to the clinical laboratory for processing and examination.

Clinical safety labs will be performed on Day 1, 8, 15, 29, and 36. If they are abnormal, they will be assessed, documented, reported, and followed until the lab returns to the subject's baseline value or has stabilized. Clinical safety laboratory parameters to be evaluated will include WBC, Hgb, platelet count, ANC, sodium, potassium, creatinine and ALT. BUN will be obtained only if creatinine is above the normal range. These evaluations will be performed by the local (clinical) laboratory. Venous blood will be collected for safety laboratory evaluations and performed by the local (clinical) laboratory for early termination visits or unscheduled visits occurring within 28 days post second study vaccination.

8.2.2. Special Assays or Procedures

Immunogenicity testing will include the following (post first vaccination):

- Humoral responses by ELISA and ADCC at Day 1, 8, 15, 22, 29 and 36.
- Humoral response by EBOV GP Neut at Day 1, 8 and 15.
- Cell-mediated immunity assays (EBOV GP T cell response) at Day 1, 8, 15, 22, and 36.
- Cell-mediated immunity assays (ChAd3 vector T cell response) at Day 1, 8, 15, 22 and 36.
- Plasmablasts (5), BCR repertoire analysis (11), CD8 tetramer staining and activation marker analysis at Day 1, 8 and 15.
- Cytokine/chemokine measurement at Day 1, 2, 3, 4, 6, 8, 9, and 15.
- Transcriptomics/Gene expression PBMCs at Day 1, 2, 3, 4, 6, 8, 9 and 15.

- Characterization of antigen-specific BCR as measured by yeast display on day 36.

Venous blood samples will also be collected for future use. 8 mL will be collected on Day 1, 8, 15, 22 and 36 post first vaccination. 42 ml will be collected on Day 182 post first vaccination. Collection of future use samples is a condition of participation in this study.

The volume (ml) of venous blood to be collected for immunogenicity assays and future research is presented in Table 2.

Table 2: Blood Volumes (ml)

Study Visit (V)	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**US**	**ED**
Study Day (from Vaccine 1)	**< -29**	**1**	**2**	**3**	**4**	**6**	**8**	**9**	**15**	**22**	**29**	**36**	**76**	**106**	**182**		
Screening Lab	14.2																
Clinical Safety Evaluations		6.5					6.5		6.5		6.5	6.5				6.5^	6.5^
High Resolution HLA tying for Class 1 (A, B, C locus)		6.0															
Humoral response – ELISA (Op 2)		10.0					10.0		10.0	10.0	10.0	10.0					10.0 ^
Humoral response – ADCC (Op 7)																	
Humoral Response, EBOV GP Neut Antibody (Op7a)																	
EBOV GP T cell responses (Op 3)		24.0					24.0		24.0	24.0		24.0					
ChAd3 vector T cell responses (Op 4)																	
Plasmablasts (Op 5, 11)		32.0					32.0		32.0								
Cd8 Tetramer Staining (Op 9)		8.0					8.0		8.0								8.0*
Activation Marker Analysis (Op 10)																	
Exploratory cytokine/ chemokine (Op 6)		5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0								
Transcriptomics PMBCs (Op 8)		16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0								
Yeast Display												16.0					
Future Use		8.0					8.0		8.0	8.0		8.0			42.0		
Total per visit	14.2	115.5	21.0	21.0	21.0	21.0	109.5	21.0	109.5	42.0	16.5	64.5	0.0	0.0	42.0	6.5	14.5-24.5
Running Total	14.2	129.7	150.7	171.7	192.7	213.7	323.2	344.2	453.7	495.7	512.2	576.7	576.7	576.7	618.7	N/A	N/A

^ *to be collected if visit occurs prior to or on Day 36 (post first vaccination)*
* *to be collected if visit occurs prior to or on Day 15 (post first vaccination)*

8.2.3. Specimen Preparation, Handling, and Shipping

8.2.3.1. Instructions for Specimen Preparation, Handling, and Storage

Instructions for specimen preparation, handling, and storage are included in the protocol-specific MOP as appropriate.

8.2.3.2. Specimen Shipment

Specimen shipment will occur when all specimens are available following all applicable International Air Transport Association (IATA) requirements and according to the specifics for storage temperature and documentation as detailed in the local (clinical) laboratory manual and protocol-specific MOP as appropriate.

All specimens for clinical screening and safety laboratory evaluations will be transported from the participating VTEU site to the local (clinical) laboratory.

Specimens for cellular assays will be transported from the participating VTEU site to the local (research) laboratory.

Specimens for antibody assays will be shipped from the participating VTEU site to the Clinical Materials Services.

Further instructions for specimen shipment are included in the local (clinical) laboratory manual and protocol-specific MOP, as appropriate.

9 ASSESSMENT OF SAFETY

9.1. Specification of Safety Parameters

Safety will be assessed by the occurrence of:

1. Serious adverse events, and MAAEs occurring from the time of study vaccination through approximately 6 months after the first study vaccination.
2. Solicited Adverse Events – reactogenicity events occurring from the time of study vaccination through 7 days after each study vaccination:
   a) Local AEs including pain, tenderness, erythema (redness), induration (hardness/swelling), ecchymosis (bruising) and pruritis (itching).
   b) Systemic AEs including fever, feverishness (chills, shivering, sweating), fatigue (tiredness), malaise (general unwell feeling), myalgia (body aches/muscular pain), nausea, loss of appetite and headache.
3. Clinical safety laboratory adverse events occurring from the time of study vaccination through approximately 28 days after the second vaccination. Parameters to be evaluated include WBC, Hgb, platelet count, ANC, sodium, potassium, Creatinine, BUN (BUN will be obtained only if creatinine is above the normal range), and ALT.
4. Unsolicited Adverse Events – study vaccine-related non-serious adverse events occurring from the time of study vaccination through approximately 28 days after the second study vaccination.

9.2. Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1. Adverse Events

Adverse Event: ICH E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

AEs, including solicited local (injection site) and systemic (subjective and quantitative) reactions, will be captured on the appropriate data collection form and eCRF. Information to be collected for unsolicited non-serious AEs includes event description, date of onset, assessment of severity, relationship to study product and alternate etiology (assessed only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as an investigator), date of resolution, seriousness and outcome. AEs occurring during the trial collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if the severity of any pre-existing medical condition increases, it should be recorded as an AE.

All AEs must be graded for severity and relationship to study product (see definitions). Adverse events characterized as intermittent require documentation of onset and duration of each episode. The start and stop date of each reported AE will be recorded on the appropriate data collection form and eCRF.

Severity of Event: AEs will be assessed by the investigator using a protocol-defined grading system (toxicity table included as an appendix). For events not included in the protocol-defined grading system, the following guidelines will be used to quantify severity:

- Mild (Grade 1): Events require minimal or no treatment and do not interfere with the subject's daily activities.
- Moderate (Grade 2): Events result in a low level of inconvenience or concern with therapeutic measures. Moderate events may cause some interference with functioning and daily activities.
- Severe (Grade 3): Events interrupt the subject's daily activities and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

Relationship to Study Products: The assessment of the relationship of an AE to the administration of study product is made only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as an investigator based on all available information at the time of the completion of the eCRF. Whether the AE is related or not, is not a factor in determining what is or is not reported in this trial. If there is any doubt as to whether a clinical observation is an AE, the event should be reported.

In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

Medically Attended Adverse Event (MAAE): For each unsolicited AE experienced, the subject will be asked if he/she had received medical attention, defined as hospitalization, an emergency room visit, or an otherwise unscheduled visit to or from medical personnel for any reason. AEs characterized by such unscheduled medical care will be designated as MAAEs.

9.2.2. Reactogenicity

Reactogenicity events are AEs that are common and known to occur following administration of this type of study vaccine. The following Toxicity Grading Scales will be used to grade solicited local (injection site) and systemic (subjective and quantitative) reactions:

Table 3: Local (Injection Site) Reactogenicity Grading

Local (Injection Site) Reaction	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Pain – experienced without touching the injection site (spontaneous discomfort)	Subject is aware of pain, but it does not interfere with daily activity, **and** no pain medication is taken	Subject is aware of pain; there is interference with daily activity **or** it requires repeated use of a non-narcotic pain reliever for >24 hours	Subject is aware of pain, **and** it prevents daily activity or requires any use of a prescription medication.
Tenderness – hurts only when injection site is touched or the arm is moved	The area immediately surrounding the injection site hurts only when touched or with arm motion, **and** it does **not** interfere with daily activity	The area immediately surrounding the injection site hurts when touched or with arm motion, **and** it interferes with daily activity	The area immediately surrounding the injection site hurts when touched or with arm motion, **and** it prevents daily activity
Erythema (Redness)*	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity
Induration (Hardness/Swelling)*	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity

Local (Injection Site) Reaction	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Ecchymosis (Bruising)*	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity
Pruritus (Itching)	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity

* Will also be measured in mm but size will not be used as halting criteria.

Ecchymosis (bruising), erythema (redness) and induration (hardness)/swelling as analyzed by measurement will be graded as follows:

Table 4: Local (Injection Site) Reactogenicity Measurements

Local (Injection Site) Reaction	Small	Medium	Large
Ecchymosis (Bruising)*	<20 mm	20 mm – 50 mm	>50 mm
Erythema (Redness)*	<20 mm	20 mm – 50 mm	>50 mm
Induration (Hardness)/Swelling*	<20 mm	20 mm – 50 mm	>50 mm

* Will not be used as halting criteria.

Table 5: Subjective Systemic Reactogenicity Grading

Systemic (Subjective)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Feverishness (chills/shivering/sweating)	noticeable but does not interfere with daily activity	interferes with daily activity	Significant interference, prevents daily activity
Malaise (General Unwell Feeling)	noticeable but does not interfere with daily activity	interferes with daily activity	Significant interference, prevents daily activity
Fatigue (Tiredness)	noticeable but does not interfere with daily activity	interferes with daily activity	Significant interference, prevents daily activity
Myalgia (Body Aches/Muscular Pain)*	noticeable but does not interfere with daily activity	interferes with daily activity	Significant interference, prevents daily activity
Headache	noticeable but does not interfere with daily activity	Any use of pain reliever or interferes with daily activity	Significant interference, prevents daily activity, or requires any use of a prescription medication

Systemic (Subjective)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Nausea	noticeable but does not interfere with daily activity	interferes with daily activity	Significant interference, prevents daily activity
Loss of Appetite	noticeable but does not interfere with daily activity	interferes with daily activity	Significant interference, prevents daily activity

* Not at injection site.

Oral temperature[#] will be graded as follows:

Table 6: Quantitative Systemic Reactogenicity Grading

Systemic (Quantitative)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Fever* - oral[†]	37.8°C – 38.4°C 100.00°F – 101.1°F	38.5°C – 38.9°C 101.2°F – 102.0°F	>38.9°C >102.0°F

[#] Oral temperature assessed on Day 1 prior to study vaccination will be considered as baseline.
* A fever can be considered not related to the study product if an alternative etiology can be documented.
† Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

Pulse and blood pressure[#] will be graded as follows:

Table 7: Blood Pressure and Pulse Grading

Physiologic Parameter	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Bradycardia - beats per minute	45-46	40 – 44	<40
Tachycardia - beats per minute	106 – 130	131 – 155	>155
Hypotension (systolic) mmHg	80 – 84	75 – 79	<75
Hypotension (diastolic) mmHg	50 – 54	45 – 49	<45
Hypertension (systolic) mmHg	151 – 155	156 – 160	>160
Hypertension (diastolic) mmHg	96 – 100	101 – 105	>105

[#] Pulse and blood pressure assessed on Day 1 prior to study vaccination will be considered as baseline.

Clinical safety laboratory results will be graded as follows:

Table 8: Clinical Safety Laboratory Adverse Event Grading (Hematology)

Hematology	Clinical Laboratory Reference Range	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
WBC K/mcL (Decrease, 18 to <21 years)	4.5 – 13.0	2.5 – 4.4	1.5 – 2.4	<1.5
WBC K/mcL (Decrease, ≥ 21 years)	4.5 – 11.0	2.5 – 4.4	1.5 – 2.4	<1.5
WBC K/mcL (Increase 18 to <21 years)	4.5 – 13.0	13.1 – 15.0	15.1 – 20.0	>20.0
WBC K/mcL (Increase ≥ 21 years)	4.5 – 11.0	11.1 – 15.0	15.1 – 20.0	>20.0
Hgb g/dL (Decrease) (Female)	11.7 – 15.7	10.1 – 11.6	8.5 – 10	<8.5
Hgb g/dL (Decrease) (Male)	13.3 – 17.7	11.0 – 13.2	9.5 – 10.9	<9.5
Platelet count K/mcL (Decrease)	135 - 466	125 – 134	100 – 124	<100
Platelet count K/mcL (Increase)	135 - 466	467 - 517	518 – 750	>750
Absolute Neutrophil Count, K/mcL* (18 to < 21 years)	1.80 – 8.00	1.5-<1.8	1.0-<1.5	<1.0
Absolute Neutrophil Count, K/mcL* (≥ 21 years)	1.80 – 7.70	1.5-<1.8	1.0-<1.5	<1.0
Absolute Neutrophil Count, K/mcL - Benign Ethnic Neutropenia*	≥ 0.8	0.6 – 0.7	0.4 -- 0.5	< 0.4

* ANC for subjects that are of African American and Middle Eastern descent may have values as low as 0.8 x K/mcL. Subjects of this descent must have an ANC ≥ 0.8 K/mcL to be eligible to participate in the study if all other study criteria are met.

Table 9: Clinical Safety Laboratory Adverse Event Grading (Chemistry)

Chemistry	Clinical Laboratory Reference Range	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
ALT unit/L (Increase)	≤ 49	50 - 123	124 - 245	> 245
Creatinine mg/dL (Increase - Female)	0.50–0.80	0.81–1.70	1.71–2.00	>2.00
Creatinine mg/dL (Increase - Male)	0.60–1.10	1.11 –1.70	1.71 2.00	>2.00
Sodium, low, mmol/L	136 – 145	130 - 135	123-129	<123
Sodium, high, mmol/L	136 - 145	146 - 150	151-157	>157
Potassium, high, mmol/L	3.5 – 5.1	5.2 - 6.0	6.1-6.5	>6.5
Potassium, low, mmol/L	3.5 – 5.1	3.0 - 3.4	2.5-2.9	<2.5
Blood Urea Nitrogen (BUN) mg/dL	9.00 – 23.00	24 - 26	27-31	>31

9.2.3. Serious Adverse Events

Serious Adverse Event (SAE): An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death,
- a life-threatening adverse event[1],
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

[1] Life-threatening adverse event. An adverse event is considered "life-threatening" if, in the view of either the site principal investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event, had it occurred in a more severe form, might have caused death.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology.
- Recorded on the appropriate SAE data collection form and eCRF.
- Followed through resolution.
- Reviewed and evaluated by an Independent Safety Monitor (ISM) (as deemed necessary), the SMC (periodic review unless related), DMID, and the IRB.

9.2.4. Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

The site principal investigator or appropriate sub-investigator is responsible for recording all AE/SAEs/MAAEs that are observed or reported during this trial, regardless of the relationship to study product. AE/SAEs/MAAEs, abnormal laboratory test values, or abnormal clinical findings will be collected, assessed, documented, reported, and followedappropriately.

For baseline laboratory results that are abnormal according to the local laboratory reference range and fall within Grade 1 toxicity table range, these will not be considered laboratory adverse event (AE) and will thus not be graded. However, if baseline clinical labs fall within Grade 1 range, then a laboratory AE is reported only if the value changes such that it falls into Grade 2 or higher when subsequent safety laboratory testing is done.

9.3. Reporting Procedures

Solicited local and systemic AEs will be documented and reported from the time of study vaccination through 7 days after each study vaccination.

Clinical safety laboratory adverse events will be documented and reported on Days 1, 8, 15, 29 and 36. The clinical safety laboratory tests will be collected prior to vaccination on Days 1 and 8. Additional safety labs will be collected and AEs reported if the subject returns to the clinic for re-evaluation of laboratory adverse events until resolution.

Unsolicited non-serious AEs will be documented and reported from the time of study vaccination through approximately 28 days after the second study vaccination.

SAEs (including lab values that meet SAE criteria), MAAEs will be documented and reported from the time of study vaccination through approximately 6 months after the first study vaccination.

At any time after completion of this trial, if the site principal investigator or appropriate sub-investigator becomes aware of an SAE that is suspected to be related to study product, the site principal investigator or appropriate sub-investigator will report the event to the DMID Pharmacovigilance Group.

9.3.1. Serious Adverse Events

SAEs will be followed until resolution even if this extends beyond the study-reporting period. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

Any AE that meets a protocol-defined serious criterion must be submitted immediately (within 24 hours of site awareness) on an SAE form to the DMID Pharmacovigilance Group, at the following address:

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20817, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

In addition to the SAE form, select SAE data fields must also be entered into the DCC system (for example: Advantage EDC). Please see the protocol-specific MOP for details regarding this procedure.

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible.

The site will send a copy of the SAE report(s) to the ISM (as deemed necessary) when they are provided to the DMID Pharmacovigilance Group. The DMID Medical Monitor and DMID Clinical Project Manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID Medical Monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the site principal investigator or appropriate sub-investigator becomes aware of an SAE that is suspected to be related to study product, the site principal investigator or appropriate sub-investigator will report the event to the DMID Pharmacovigilance Group.

9.3.2. Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Following notification from the investigator, DMID, the Investigational New Drug (IND) sponsor, will report any suspected adverse reaction that is both serious and unexpected. DMID will report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event. DMID will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its IND(s) or under any principal investigator's IND(s)) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. DMID will also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. Relevant follow up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

All serious events designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

9.3.3. Other Adverse Events

N/A

9.3.4. Reporting of Pregnancy

Pregnancies occurring in study subjects will be reported via AdvantageEDC[SM] on the Pregnancy Report form. With the subject's permission, all protocol-required venous blood samples will be obtained and the subject will continue to be followed for safety for the duration of this trial. Efforts will be made to follow all pregnancies reported during the course of this trial to pregnancy outcome pending the subject's permission.

9.4. Type and Duration of Follow-up of Subjects after Adverse Events

AEs will be collected, assessed, and followed through resolution from the time of study vaccination through approximately 28 days after the second study vaccination.

SAEs and MAAEs will be collected, assessed, and followed from the time of study vaccination through resolution even if this extends beyond the trial-reporting period (approximately 6 months after the first study vaccination).

Resolution of an AE/SAE/MAAE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

Follow-up procedures, evaluations, and outcomes will be recorded on the appropriate data collection form.

9.5. Halting Rules

Further enrollment and study vaccinations will be halted for SMC review/recommendation if any of the following are reported:

- Any subject experiences a study product-related SAE from the time of the study product administration through the subject's last study visit.
- Any subject experiences laryngospasm, bronchospasm, or anaphylaxis within 1 day after administration of study product that is considered related to study product.
- Two or more subjects experience generalized urticaria (defined as occurring at more than two body parts) within 3 days after administration of study product that is considered related to study product.
- Two or more subjects experience the same grade 3 unsolicited AE (in the same MedDRA High Level Term) after administration of study product that is considered related to study product and not resolved or improved to lower grade within 2 days.
- Two or more subjects experience the same grade 3 laboratory adverse event that is considered related to study product.

This trial will also be halted for SMC review/recommendation if, within 7 days after administration of any study vaccination, any of the following occurs:

- Two or more subjects experience the same grade 3 solicited local adverse event that is considered related to study product and not resolved or decreased to lower grade within 2 days.
- Two or more subjects experience the same grade 3 solicited systemic adverse event that is considered related to study product and not resolved or decreased to lower grade within 2 days.

Grading scales for solicited local (application site) and systemic (subjective and quantitative) AEs are included in Section 9.2.2.

Grading scales for clinical safety laboratory adverse events are included in Section 9.2.2.

If any of the halting rules are met following any subject receipt of any study vaccination, then this trial will not continue with the remaining enrollments or study vaccinations without a review by and recommendation from the SMC to proceed.

DMID retains the authority to suspend additional enrollment and study interventions/ administration of study product during the entire trial, as applicable.

9.6. Safety Oversight (ISM plus SMC)

9.6.1. Independent Safety Monitor (ISM)

An ISM is a physician with relevant expertise whose primary responsibility is to provide to DMID an independent safety assessment in a timely fashion. Participation is for the duration of the DMID study and is a voluntary position that does not receive payment. The ISM must meet the requirements of the NIAID conflict of interest policy.

The ISM:

- Is in close proximity to the study site and has the authority and ability to readily access study participant records in real time.
- May be a member of the participating institution's staff but preferably be from a different organizational group within the institution.
- Should not be in a direct supervisory relationship with the investigator.
- Should have no direct involvement in the conduct of the study.

The ISM will:

- Sign a Conflict of Interest (COI) certification at the time they are asked to participate and provide updates to this information as needed.
- Receive reports of Serious Adverse Events (SAEs) from the site investigator and will be notified by email when DMID is notified of the SAE.
- Evaluate the SAE and report their clinical assessment to DMID, through DMID-CROMS SOCS in a timely manner using the attached report form and email the report to DMID-CROMS SOCS.
- Communicate with the investigator at the participating site as needed.
- Review additional safety related events at the request of DMID.
- Provide additional information to DMID and/or the SMC by teleconference as requested.

9.6.2. Safety Monitoring Committee (SMC)

This clinical study will utilize an SMC, which is an independent group of experts that advises DMID. The primary responsibility of the SMC is to monitor subject safety. The SMC is external to the DMID and comprises at least 3 voting members. The SMC will consist of members with appropriate Phase 1 study expertise to contribute to the interpretation of the data from this trial. Its activities will be delineated in a SMC charter that will describe the membership, responsibilities, and the scope and frequency of data reviews. The SMC will operate on a conflict-free basis independently of the study team. The DMID or the SMC may convene ad hoc meetings of the SMC according to protocol criteria or if there are concerns that arise during the study.

The SMC will review the safety data at the following milestones:

- Organizational meeting (prior to start of the study)
- Scheduled Data Review meetings
- An SMC Ad Hoc meeting will be convened when a halting rule is met, or at the request of the investigator and/or DMID if there are safety concerns during the course of the study.
- Final review meeting, to be conducted 6 to 8 months after clinical database lock to review the cumulative unblinded safety data for the study. The data will be provided in a standard summary format. The SMC may be asked to provide recommendations in response to questions posed by the DMID.

The SMC will operate under the rules of a DMID-approved charter that will be written at the organizational meeting of the SMC. At this time, each data element that the SMC needs to assess

will be clearly defined. Procedures for SMC reviews/meetings will be defined in the charter. The SMC will review applicable data to include, but not limited to, study progress and participant, clinical, safety, and reactogenicity data. Reports may include enrollment and demographic information, medical history, concomitant medications, physical assessments, clinical laboratory values, dosing compliance, and solicited and unsolicited AE/SAEs/MAAEs. Additional data may be requested by the SMC, and interim statistical reports may be generated as deemed necessary and appropriate by DMID. The SMC may receive data in aggregate and presented by cohort. The SMC may also be provided with expected and observed rates of the expected AEs in an unblinded fashion, and may request the treatment assignment be unblinded for an individual subject if required for safety assessment. The SMC will review grouped and unblinded data in the closed session only. The SMC will meet and review this data at scheduled time points or ad hoc as needed during this trial as defined in the SMC charter. As an outcome of each review/meeting, the SMC will make a recommendation as to the advisability of proceeding with study vaccinations (as applicable), and to continue, modify, or terminate this trial.

DMID or the SMC chair may convene the SMC on an ad hoc basis according to protocol criteria or if there are immediate concerns regarding observations during the course of this trial. The DMID Medical Monitor is empowered to stop enrollment and study vaccinations if adverse events that meet the halting criteria are reported. The DMID Medical Monitor and the ISM (as deemed necessary) will be responsible for reviewing SAEs in real time. The SMC will review SAEs on a regular basis and ad hoc during this trial.

10 CLINICAL MONITORING

10.1. Site Monitoring Plan

Site monitoring is conducted to ensure that the human subjects' protections, study and laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, ICH/GCP guidelines and applicable regulations, and that this trial is conducted in accordance with the protocol, protocol-specific MOP and applicable sponsor standard operating procedures. DMID, the sponsoring agency, or its designee will conduct site-monitoring visits as detailed in the clinical monitoring plan.

Site visits will be made at standard intervals as defined by DMID and may be made more frequently as directed by DMID. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, eCRFs, informed consent forms, medical and laboratory reports, and protocol and GCP compliance. Site monitors will have access to each participating VTEU site, study personnel, and all study documentation according to the DMID-approved site monitoring plan. Study monitors will meet with site principal investigators to discuss any problems and actions to be taken and document visit findings and discussions.

11 STATISTICAL CONSIDERATIONS

This is a Phase 1, randomized, double-blind trial of 60 males and non-pregnant females, 18-45 years old, inclusive, who are in good health, meet all eligibility criteria, and do not meet any exclusion criteria. This trial is designed to assess the safety, reactogenicity and immunogenicity of a *Zaire ebolavirus* (ChAd3-EBO-Z) vaccine for the prevention of Ebola virus disease. ChAd3-EBO-Z will be administered intramuscularly on Day 1 followed by either placebo (Group 1), ChAd3-EBO-Z (Group 2), or MVA- BN®-Filo (Group 3) at Day 8. The clinical trial includes an exploratory systems biology component to identify changes in gene expression that are associated with adaptive humoral and/or cellular immune responses.

11.1. Study Hypotheses

This Phase I study is not designed to test a formal null hypothesis. The sample size for this study was selected to obtain preliminary estimates of vaccine safety and immunogenicity. The clinical trial includes an exploratory systems biology component to identify changes in gene expression, and cytokines that are associated with adaptive humoral and/or cellular immune responses. These systems biology results are expected to generate hypotheses to be verified in future studies.

11.2. Sample Size Considerations

See Section 11.1 and Section 4.

11.3. Planned Interim Analyses

11.3.1. Interim Safety Review

An interim safety review may include enrollment and demographic information, medical history, concomitant medications, physical assessments, dosing compliance, protocol adherence, clinical laboratory values, and solicited and unsolicited AEs. Additional data may be requested by the SMC, and interim safety reports may be generated as deemed necessary and appropriate by DMID. The SMC will receive data in aggregate. The SMC may request the treatment group be unblinded for an individual subject if required for safety assessment. The SMC will review grouped and unblinded data in the closed session only. The SMC will meet and review this data at scheduled time points or ad hoc as needed during this trial as defined in the SMC charter. As an outcome of each review/meeting, the SMC will make a recommendation as to the advisability of proceeding with study vaccinations (as applicable), and to continue, modify, or terminate this trial.

11.3.2. Interim Immunogenicity Review

An interim immunogenicity review is not planned.

11.4. Final Analysis Plan

The final CSR will be completed after the last subject's last visit is completed, and the final clinical database including all long-term safety follow-up data is cleaned, monitored and locked. The primary CSR will include results for all primary and secondary endpoint data as well as exploratory EBOV GP pseudovirion neutralization assay data. Additional results for exploratory endpoint data will be included as one or more addenda to the primary CSR. A formal Statistical Analysis Plan that specifies all planned analyses will be developed and finalized prior to the final clinical database lock.

11.4.1. Analysis Populations

The Safety Analysis population includes all subjects who received at least one study vaccination.

The intent-to-treat (ITT) population includes all subjects who received at least one study vaccination and contributed both pre- and at least one post-study vaccination blood samples for immunogenicity testing for which valid results were reported. Subjects will be analyzed according to the study group to which they were randomized.

The per protocol (PP) population includes all subjects in the ITT subset with the following exclusions:

- Data from all available visits for subjects found to be ineligible at baseline.
- Data from all visits subsequent to major protocol deviations, such as:
    - Second study vaccination not received,
    - Receipt of non-study licensed live vaccine within 30 days prior to or after each study vaccination,
    - Receipt of non-study licensed inactivated vaccine within 14 days prior to or after each study vaccination,
    - Receipt of oral or parenteral (including intraarticular) corticosteroids of any dose within 30 days prior to study vaccination.
    - Receipt of high-dose inhaled corticosteroids within 30 days prior to study vaccination.
    - Receipt of chronic (defined as more than 14 days) immunosuppressants or other immune modifying drugs within 6 months of receipt of study vaccine.
- Data from any visit that occurs outside the pre-defined protocol window.
- In the case of mis-randomization, subjects will be analyzed according to the study product actually received.

11.4.2. Safety Data

Safety data will be summarized for the Safety Analysis Population. Pre-second vaccination, Study Groups 1, 2, 3 will be analyzed jointly as one study group as all three groups received the same vaccine at Day 1.

Solicited AEs will be summarized by severity for each day after each study vaccination (Days 1 through 7 days post-vaccination) and as the maximum severity over all days after each vaccination. The maximum severity of local and systemic reactions will be determined for each subject and summarized by study group following each vaccination (as well as post any vaccination) and the resulting number, percentage (observed rate) of subjects and associated exact 95% confidence interval (CI) will be summarized by severity grade (none, mild, moderate, severe) and symptom. Summaries will also be provided for any systemic symptoms, for any local symptoms, and for any systematic or local symptoms.

Vaccine-related unsolicited AEs from the first study vaccination through 28 days post second vaccination will be coded by Medical Dictionary for Regulatory Activities (MedDRA®) for preferred term and system organ class (SOC). These AEs will be summarized as number and percentage of subjects reporting at least one event in each MedDRA® preferred term and SOC, cross tabulated by severity grade (none, mild, moderate, severe). Additionally, the number, percentage (observed rate) and exact two-sided 95% CI for subjects reporting a related unsolicited AE overall, by SOC, and by preferred term will be summarized by study group.

Clinical safety laboratory AEs from the time of first study vaccination through approximately 28 days after the second vaccination will be summarized by severity grade (none, mild, moderate, severe) for each laboratory visit. In addition, the maximum severity over all post-vaccination visits will be summarized by study visit and by severity grade (none, mild, moderate, severe) using percentages and exact two-sided 95% CIs.

The number of SAEs and vaccine-related Medically Attended Adverse Events (MAAEs) through 6 months post first vaccination will be reported by detailed listings showing the event description, preferred term and SOC, relevant dates (study vaccinations and AEs), severity, vaccine-relatedness, and outcome for each event.

11.4.3. Secondary Immunogenicity Data

Immunogenicity data summaries and analysis will be presented for both the ITT and PP populations. Pre-second vaccination, Study Groups 1, 2, 3 will be analyzed jointly as one study group as all three study groups received the same vaccine at Day 1.

Seroconversion for anti-EBOV GP ELISA (defined as EBOV GP ELISA titer > 50 if baseline (Day 1) titer ≤ 50 or fold rise of > 4-fold as compared to baseline if the baseline titer > 50), will be summarized for each post-vaccination study visit day (Day 8, 15, 22, 29 and 36) and study

group using percentages and exact two-sided 95% CIs. Per-visit seroconversion percentages and CIs will be visualized across study visit days using time trend plots.

Anti-EBOV GP ELISA titer at Day 1, 8, 15, 22, 29 and 36 will be summarized by calculating the geometric mean (GMT) titer and corresponding 95% CIs for each study group and day by transforming individual titer results to a logarithmic scale, assuming asymptotic normality conditions were satisfied on this scale, computing the mean and 95% CI then converting back to the original scale. Per-visit GMTs and CIs will be visualized across study visit days using time trend plots.

Change in Anti-EBOV GP ELISA titer at Day 8, 15, 22, 29 and 36 compared to baseline (Day 1) will be summarized by calculating the geometric mean fold rise (GMFR) and corresponding 95% CIs for each study group and day by dividing the post-vaccination titer for each subject by the respective pre-vaccination titer, transforming ratios to a logarithmic scale, assuming asymptotic normality conditions were satisfied on this scale, computing the mean and 95% CI then converting back to the original scale. Per-visit GMFR and CIs will be visualized across post-vaccination study visit days using time trend plots.

11.4.4. Exploratory Data Analyses

Pre-second vaccination, Study Groups 1, 2, 3 will be analyzed jointly as one study group as all three study groups received the same vaccine at Day 1.

GMT of neutralizing antibodies to EBOV GP at Day 8 and 15 and GMFR in titer on Day 8 and 15 compared to baseline (Day 1) and associated 95% CIs will be calculated and summarized as described in Section 11.4.3. Significant differences in neutralizing antibodies to EBOV GP or Anti-EBOV GP ELISA titer between study groups for each post-second vaccination time points will be evaluated using a two-sided Welch's T-test using an individual alpha of 0.05. Differences in Anti-EBOV GP ELISA-based seroconversion at post-second vaccination time points will be assessed using a two-sided Fishers' exact test using an individual alpha of 0.05.

Baseline fold changes for[35] percentage of killed cells[36] percentage of activated CD4+ and CD8+ T cells[37], cytokine concentrations[38], percentage of EBOV GP-specific cytokine secreting CD4+ and CD8+ T cells expression one, two, or multiple makers[39], percentage of EBOV GP-specific CD4+ and CD8+ T cells[40], percentage of ChAd3-specific cytokine secreting CD4+ and CD8+ T cells[41], percentage of CD19+CD27+CD38+ plasmablasts will be summarized using minimum, Q1, median, Q3, maximum, and 95% CI of the median. The 95% CI of the median fold change from baseline will be determined by using the bootstrap method and visualized using time trend plots. To identify features that show a differential response from baseline, a two-sided Wilcoxon signed-rank test will be carried out for each study group using an individual alpha of 0.05. Significant differences in fold changes between study groups for post-second vaccination time

points will be evaluated using a two-sided Wilcoxon rank-sum test using an individual alpha of 0.05.

For the transcriptomics exploratory analysis, RNA-Seq data will be pre-processed by removing adapters and low-quality reads and mapping sequences to the latest human reference genome using splice-aware alignment software such as *HISAT2*[35]. Gene expression quantification will be carried out by using the *Subread* software[36] using the latest Ensembl[37] gene model annotations as a reference. Systematic differences in sequence coverage between samples will be accounted for using the TMM method[38] as implemented in the *edgeR*[39] R package. Principal component analysis, non-metric multidimensional scaling, and hierarchical clustering analysis will be used to identify potential global outliers and systematic batch effect. Negative binomial models as implemented in *edgeR*[39] will be used to identify genes for each study group and post-vaccination day that were differentially expressed (DE) compared to pre-vaccination as well as genes that were DE between study groups. If the analysis reveals a systematic batch effect that is not associated with study group, it will be accounted for by adding a batch blocking factor as part of the negative binomial models. To control for testing multiple genes, the false-discovery rate (FDR) based on the Benjamini-Hochberg procedure[40] as implemented in the *p.adjust* R function will be applied. Genes with a FDR-value < 0.05 and a fold change of greater or equal to 1.5-fold (up or down regulation) will be deemed to be significantly DE. Depending on observed treatment effects, this fold change cut off may be revised. The *pvclust*[41] R package will be applied to identify co-expressed DE gene clusters and cluster time trends will be visualized. Pathway enrichment analysis based on the latest MSigDB[42] and KEGG[43] databases as well as Blood Transcription Modules[44] accounting for gene length bias will be carried out using the implementation provided by the *goseq*[45] R package. Pathway maps color-coded by treatment effect will be provided for significantly enriched KEGG pathways. Regularized canonical correlation analysis and regularized linear regression analysis as implemented in the mixOmics[46] and glmnet[47] R packages, respectively will be utilized to identify gene expression changes that are associated with adaptive humoral and/or cellular immune response endpoints. In both cases, cross-validation will be used to select optimal models.

Please see the separate document "**Statistical Analysis Plan**" for additional information on exploratory endpoint analyses.

11.4.5. Missing Values and Outliers

All attempts will be made to collect all data per protocol.

As missing data are expected to be minimal, no imputation will be performed for missing values. Any data point that appears to be erroneous or inexplicable based on clinical judgment will be investigated as a possible outlier. If data points are identified as outliers, sensitivity analyses will be performed to examine the impact of including or excluding the outliers. Any substantive differences in these analyses will be reported.

For the transcriptomics endpoint, extreme data points will be identified by inspecting global patterns in the data. Experimental meta-data will be examined to see whether the outlying pattern can be attributed to experimental error such as sample mislabeling. Excluded outliers will be documented in the CSR. Due to the complexity, no sensitivity analysis will be carried out for the transcriptomics endpoints.

Please see the separate document **"Statistical Analysis Plan"** for additional information.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Each participating site will maintain appropriate medical and research records in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. Each site will permit authorized representatives of the DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. These representatives will be permitted access to all source data and source documents, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subjects' memory aid or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial.

Interview of subjects is sufficient for obtaining medical history.

13 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, each participating site and its subcontractors are responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The site principal investigator will provide direct access to all study-related sites, source data/data collection forms, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The site principal investigator will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

The DCC will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the participating site(s) for clarification and resolution.

DMID-designated clinical monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, ICH/GCP guidelines, and the applicable regulatory requirements. Clinical monitoring reports will be submitted to DMID.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1. Ethical Standard

The site principal investigator (PI) will ensure that this trial is conducted in full conformity with principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research (National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research [April 18, 1979]) and codified in 45 CFR 46, 21 CFR 50 and 21 CFR 56, as applicable. The PI will also ensure conformity with ICH E6 Good Clinical Practice, and applicable federal regulations, guidance, and guidelines for Good Clinical Practice and Clinical Trials with humans.

14.2. Institutional Review Board

The site principal investigator will obtain IRB approval for this protocol to be conducted at his/her research site(s), and send supporting documentation to the DMID before initiating recruitment of subjects. The investigator will submit applicable information to the IRB/IEC on which it relies for the review, to conduct the review in accordance with 45 CFR 46, ICH E6 GCP, and as applicable, 21 CFR 56 (Institutional Review Boards) and 21 CFR 50 (Protection of Human Subjects), other federal, state, and local regulations. The IRB/IEC must be registered with Office of Human Research Protection [*OHRP-only* or *OHRP/FDA*] as applicable to the research. DMID must receive the documentation that verifies IRB/IEC-approval for this protocol, associated informed consent documents, and upon request any recruitment material and handouts or surveys intended for the subjects, prior to the recruitment and enrollment of subjects.

Any amendments to the protocol or consent materials will be approved by the IRB/IEC before they are implemented. IRB/IEC review and approval will occur at least annually throughout the enrollment and follow-up of subjects, and may cease if annual review is no longer required by applicable regulations. The investigator will notify the IRB/IEC of deviations from the protocol and reportable SAEs, as applicable to the IRB/IEC policy.

Each institution engaged in this research will hold a current Federalwide Assurance (FWA) issued by the Office of Human Research Protection (OHRP) for federally funded research.

14.3. Informed Consent Process

Informed consent is a process that is initiated prior to an individual agreeing to participate in a trial and continuing throughout the individual's trial participation. Before any study procedures are performed, informed consent will be obtained and documented. Subjects will receive a concise and focused presentation of key information about the clinical trial, verbally and with a written consent form. The explanation will be organized, and presented in lay terminology and language that facilitates understanding why one might or might not want to participate.

An investigator or designee will describe the protocol to potential subjects face-to-face. The key information about the purpose of the study, the procedures and experimental aspects of the study, risks and discomforts, any expected benefits to the subject, and alternative treatment will be presented first to the subject.

Subjects will also receive an explanation that the trial involves research, and a detailed summary of the proposed study procedures and study interventions/products. This will include aspects of the trial that are experimental, the probability for random assignment to treatment groups, any expected benefits, all possible risks (including a statement that the particular treatment or procedure may involve risks to the subject or to the embryo or fetus, if the subject is or may become pregnant, that are currently unforeseeable), the expected duration of the subject's participation in the trial, alternative procedures that may be available and the important potential benefits and risks of these available alternative procedures.

Subjects will be informed that they will be notified in a timely manner if information becomes available that may be relevant to their willingness to continue participation in the trial. Subjects will receive an explanation as to whether any compensation and any medical treatments are available if injury occurs, and, if so, what they consist of, or where further information may be obtained. Subjects will be informed of the anticipated financial expenses, if any, to the subject for participating in the trial, as well as any anticipated prorated payments, if any, to the subject for participating in the trial. They will be informed of whom to contact (e.g., the investigator) for answers to any questions relating to the research project.

Information will also include the foreseeable circumstances and/or reasons under which the subject's participation in the trial may be terminated. The subjects will be informed that participation is voluntary and that they are free to withdraw from the study for any reason at any time without penalty or loss of benefits to which the subject is otherwise entitled.

The extent of the confidentiality of the subjects' records will be defined, and subjects will be informed that applicable data protection legislation will be followed. Subjects will be informed that the monitor(s), auditors(s), IRB, NIAID, and regulatory authority(ies) will be granted direct access to the subject's original medical records for verification of clinical trial procedures and/or data without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations, and that, by signing a written informed consent form, the subject is authorizing such access.

Subjects will be informed that records identifying the subject will be kept confidential, and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available and, if the results of the trial are published, the subject's identity will remain confidential. Subjects will be informed whether private information collected from this research and/or specimens will be used for additional research, even if identifiers are removed.

Subjects will be allowed sufficient time to consider participation in this research trial, and have the opportunity to discuss this trial with their family, friends or legally authorized representative, or think about it prior to agreeing to participate.

Informed consent forms will be IRB-approved and subjects will be asked to read and review the consent form. Subjects must sign the informed consent form prior to starting any study procedures being done specifically for this trial.

Once signed, a copy of the informed consent form will be given to the subject(s) for their records. The subject(s) may withdraw consent at any time throughout the course of the trial. The rights and welfare of the subject(s) will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Study personnel may employ recruitment efforts prior to obtaining study consent if a patient-specific screening consent is on record or if the IRB has agreed that chart review is allowed without a fully executed screening consent. In cases where there is not a patient-specific screening consent on record, site Clinical staff may pre-screen via chart review and refer potential subjects to the Research staff. Research staff would obtain written consent per the standard informed consent process before conducting protocol-specific screening activities.

New information will be communicated by the site principal investigator to subjects who consent to participate in this trial in accordance with IRB requirements. The informed consent document will be updated and subjects will be re-consented per IRB requirements, if necessary. Subjects will be given a copy of all informed consent forms that they sign.

14.3.1. Informed Consent/Assent Process (in Case of a Minor)

N/A

14.4. Exclusion of Women, Minorities, and Children (Special Populations)

This trial will be inclusive of all adults who meet the Subject Inclusion Criteria (see Section 5.1) and do not meet the Subject Exclusion Criteria (see Section 5.2), regardless of religion, sex, or ethnic background. Should the outcome of this trial be deemed acceptable, additional trials may be initiated including those in other populations.

It is unknown if the vaccine poses any risks to an unborn child. Special populations, e.g., non-English speakers, children, illiterate or non-writing individuals and vulnerable populations will not be enrolled in this study. The rationale for this is that this is an exploratory phase I study in healthy individuals that requires intensive visits and blood draws, which is most appropriately conducted in healthy, literate adults.

14.5. Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality includes documentation, investigation data, subject's clinical information, and all other information generated during participation in the study. No information concerning the study or the data generated from the study will be released to any unauthorized third party without prior written approval of the DMID and the subject. Subject confidentiality will be maintained when study results are published or discussed in conferences. The study monitor or other authorized representatives of the sponsor or governmental regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

All records will be kept locked and all computer entry and networking programs will be carried out with coded numbers only and with password protected systems. All non-clinical specimens, evaluation forms, reports, and other records that leave the site will be identified only by a coded number.

To protect privacy, we have received a Certificate of Confidentiality. With this Certificate, the researchers cannot be forced to release information that may identify the research subject, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify the subject, except as explained below.

The Certificate cannot be used to resist a demand for information from personnel of the United States (US) Government that is used for auditing or evaluation of federally funded projects, like this study, or for information that must be released in order to meet the requirements of the Federal Food and Drug Administration (FDA).

A Certificate of Confidentiality does not prevent the subject from voluntarily releasing information about themselves or their involvement in this research. If any person or agency obtains a written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

The Certificate of Confidentiality does not prevent the researchers from reporting without the subject's consent, information that would identify the subject as a participant in the research project regarding matters that must be legally reported including: child and elder abuse, sexual abuse, or wanting to harm themselves or others.

14.6. Study Discontinuation

If the study is prematurely terminated by the sponsor, any regulatory authority, or the investigator for any reason, the investigator will promptly inform the study subjects and assure appropriate therapy or follow-up for the subjects, as necessary. The investigator will provide a detailed written explanation of the termination to the IRB/IEC. If any subject's private information will continue to be collected for this study, the IRB/IEC must approve a consent form with the study procedures, any risks and discomforts, and applicable elements, and the investigator or designee will re-consent the subjects as approved by the IRB/IEC.

14.7. Costs, Subject Compensation, and Research Related Injuries

There is no cost to subjects for the research tests, procedures, and study product while taking part in this trial. Subjects may be compensated for their participation in this trial. Compensation will be in accordance with the local IRB's policies and procedures, and subject to IRB approval.

If it is determined by the site principal investigator that an injury occurred to a subject as a direct result of the tests or treatments that are done for this trial, then referrals to appropriate health care facilities will be provided to the subject. Study personnel will try to reduce, control, and treat any complications from this trial. Immediate medical treatment may be provided by the participating site. No financial compensation will be provided to the subject by the NIAID, NIH to the subject, or by the participating site for any injury suffered due to participation in this trial.

14.8. Future Use of Stored Specimens and Data

Residual samples are those that are left over after the study has been completed and are stored for possible use in future research studies. Future use samples are extra tube/s of blood collected and stored for possible use in future research studies. Retention of residual and future use samples and the potential for use in future research studies will be a condition of study participation. Subjects who sign the informed consent form for the study are consenting to allow the collection, storage and use of any residual samples (serum or cells derived from vemous blood samples) or future use samples for possible use in future research studies, such as examining additional immunological assessments or testing for antibodies against other viruses or bacteria.

Future use samples will be collected at Day 1, 8, 15, 22, 36 and 182. It is anticipated that approximately two 0.5 mL aliquots of plasma and 2 aliquots of 5 x 10^6 cells from each extra 8 mL venous blood sample will be available specifically for the purpose of future research at D 1, 8, 15, 22 and 36. It is anticipated that approximately ten 0.5 mL aliquots of plasma and 8 aliquots of 5 x 10^6 cells from each extra 42 mL venous blood sample will be available specifically for the purpose of future research at D 182. Future use research studies may include, but are not limited

to non-traditional immune assay development, assessing innate immune factors and the ability of ebola vaccine-induced antibodies to cross-react with other viruses.

Residual and future use samples will be encoded with a barcode label and unique tracking number to protect subject identity. Samples will be stored indefinitely at a DMID designated central storage facility. Residual samples may be shared for purposes other than per protocol analysis with investigators at participating VTEU sites and with investigators at other institutions once the clinical study report has been finalized. Future use research samples may be requested from DMID and shipped from the DMID CMS at any time.

Residual and future use samples will not be sold or used directly for production of any commercial product. Genetic tests may be performed on samples. There are no benefits to subjects in the collection, storage and subsequent use of their specimens for future research. Reports about future research done with subjects' samples will NOT be kept in their health records.

Subjects may withdraw consent for study participation at any time by notifying the study doctors or nurses in writing. However, any data from sample(s) collected prior to the withdrawn consent will not be removed including genomic data. There will be no further use of residual samples or collection and use of future use samples after consent has been withdrawn.

15 DATA HANDLING AND RECORD KEEPING

The investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue permanent ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

Copies of the electronic CRF (eCRF) will be provided for use as source data collection forms and maintained for recording data for each subject enrolled in the study. Data reported in the eCRF derived from source data collection forms should be consistent or the discrepancies should be explained.

15.1. Data Management Responsibilities

Data collection is the responsibility of the study personnel at the participating clinical study site under the supervision of the site principal investigator. During the study, the site principal investigator must maintain complete and accurate documentation for the study.

The data coordinating center (DCC) for this study will be responsible for data management, quality review, analysis, and reporting of the study data.

15.2. Data Capture Methods

Clinical (including, but not limited to, AE/SAEs/MAAEs, concomitant medications, medical history, physical assessments, and clinical laboratory values) and reactogenicity will be collected on data collection forms by study personnel then entered into eCRFs via a 21 CFR Part 11-compliant internet data entry system provided by the study data coordinating center. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

15.3. Types of Data

Data for this trial will include clinical, safety, and outcome measures (e.g., clinical laboratory values, reactogenicity, and immunogenicity data).

15.4. Timing/Reports

See Section 9.3 for further reporting details

Interim statistical reports may be generated as deemed necessary and appropriate by DMID. Safety reports may be generated for the SMC (see Section 11.3.1).

After full analysis and final reporting is complete, and upon request and DMID approval, the SDCC will provide the participating VTEU site with a summary of results by treatment arm and/or subject treatment assignments. In this regard, the participating VTEU site requesting such information to share with study subjects must do so in compliance with their respective IRB guidelines.

15.5. Study Records Retention

Study documents will be retained for a minimum of 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 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, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

15.6. Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or protocol-specific MOP requirements. The noncompliance may be either on the part of the subject, the site principal investigator, or the site personnel. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, Section 5.1.1

5.20 Noncompliance, Sections 5.20.1, and 5.20.2.

It is the responsibility of the site principal investigator and personnel to use continuous vigilance to identify and report deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID per the DCC protocol deviation reporting procedures.

All protocol deviations, as defined above, must be addressed in study subject data collection forms. A completed copy of the DMID Protocol Deviation Form must be maintained in the Regulatory File, as well as in the subject's chart. Protocol deviations must be sent to the local IRB/IEC per their guidelines. The site principal investigator and personnel are responsible for knowing and adhering to their IRB requirements.

16 PUBLICATION POLICY

All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/) an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.

Refer to:

NIH Public Access Policy, http://publicaccess.nih.gov/

NIH OER Grants and Funding, http://grants.nih.gov/grants/oer.htm

As of January 2018, all clinical trials supported by the NIH must be registered on ClinicalTrials.gov, no later than 21 days after the enrollment of the first subject. Results of all clinical trials supported by the NIH, generally, need to be submitted no later than 12 months following the primary completion date. A delay of up to 2 years is available for trials that meet certain criteria and have applied for certification of delayed posting.

As part of the result posting a copy of this protocol (and its amendments) and a copy of the Statistical Analysis Plan will be posted on ClincialTrials.gov.

For this trial the responsible party is DMID which will register the trial and post results.

The responsible party does not plan to request certification of delayed posting.

Refer to:

Public Law 110-85, Section 801, Clinical Trial Databases

42CFR11

NIH NOT-OD-16-149

17 LITERATURE REFERENCES

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18 SUPPLEMENTS/APPENDICES

Appendix A: Schedule of Study Procedures and Evaluations

Appendix B: Estimated Comparative Daily Doses - Inhaled Corticosteroids for Long-Term Asthma Control (12 years and older)

Appendix C: Acceptable Ranges of Screening Laboratory Measurements

APPENDIX A: SCHEDULE OF STUDY PROCEDURES AND EVALUATIONS

Study Visit (V)	00	01	02	03	04	05	06	07	08	09	10	11	12	13	14	**U/S[2]**	**ET[2]**
Study Day from Vaccine 1	**Day < -29 to -1**	**Day 1 Vaccine 1**	**Day 2**	**Day 3**	**Day 4**	**Day 6**	**Day 8 +2**	**Day 9**	**Day 15**	**Day 22**	**Day 29**	**Day 36**	**Day 76 +/- 7**	**Day 106 +/-7**	**Day 182 +/- 14**	-	-
Study Day from Vaccine 2							**Day 1 Vaccine 2**	**Day 2**	**Day 8 +1**	**Day 15 +2, - 1**	**Day 22 +/- 1**	**Day 29 +/- 2**				-	-
Visit Type	**Screen**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Call**	**Call**	**Clinic**	**Clinic**	**Clinic**
Obtain Informed Consent[∞]	X[†¬]	X[¬]															
Collect Demographic Information	X																
Review Eligibility Criteria	X	X[†¬1]					X[†¬]										
Medical History[@]	X	X[†¬1]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X[√]	X[†¬]	X	X	X	X	X	X	X	X	X	X				X	X
Vital Signs (oral temp, pulse, BP)[%$]	X	X[†]					X[†]									X	X
Height & Weight	X																
ECG	X	[X]	[X]	[X]	[X]	[X]	[X]†	[X]	[X]	[X]	[X]	[X]			[X]	[X]	[X]
Physical Examination	X	{X}[†]	{X}	{X}	{X}	{X}	{X}[†]	{X}	{X}	{X}	{X}	{X}			{X}	{X}	{X}
Urine or Serum Pregnancy Test^	X	X[†]					X[†]										
Urine Dipstick, Opioid testing	X																
Enrollment/Randomization		X															
Pre-administration reactogenicity assessments		X[†]					X[†]										
Study Vaccination		X[3]					X[4]										
30-minute evaluation post vax		X					X										
Distribute Memory Aid/Materials		X					X										
Review Memory Aid			X	X	X	X	X	X	X							X	X
Assessment of Adverse Events		X	X	X	X	X	X	X	X	X	X	X				X	X
Assessment of SAEs/MAAEs		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Study Visit (V)	00	01	02	03	04	05	06	07	08	09	10	11	12	13	14	**U/S[2]**	**ET[2]**
Study Day from Vaccine 1	**Day < -29 to -1**	**Day 1 Vaccine 1**	**Day 2**	**Day 3**	**Day 4**	**Day 6**	**Day 8 +2**	**Day 9**	**Day 15**	**Day 22**	**Day 29**	**Day 36**	**Day 76 +/- 7**	**Day 106 +/-7**	**Day 182 +/- 14**	-	-
Study Day from Vaccine 2							**Day 1 Vaccine 2**	**Day 2**	**Day 8 +1**	**Day 15 +2/ - 1**	**Day 22 +/- 1**	**Day 29 +/- 2**				-	-
Visit Type	**Screen**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Call**	**Call**	**Clinic**	**Clinic**	**Clinic**
Laboratory Assessments[5]																	
Screening Labs~	14.2[=&>]																
HLA typing for Class 1 (A, B, C locus)		6[†]															
Clinical Safety Evaluations*		6.5[†]					6.5[†]		6.5		6.5	6.5				6.5	6.5
Humoral response – ELISA										10	10	10					
Humoral response - ADCC		10[†]					10[†]		10								10
Humoral Response, EBOV GP Neut Antibody																	
EBOV GP T cell responses		24[†]					24[†]		24	24		24					
ChAd3 vector T cell responses																	
Plasmablasts		24[†]					24[†]		24								
Cd8 Tetramer Staining		8[†]					8[†]		8								8
Activation Marker Analysis																	
Exploratory cytokine/chemokine		5[†]	5	5	5	5	5[†]	5	5								
Transcriptomics/Gene Expression PBMCs 8)		16[†]	16	16	16	16	16[†]	16	16								
Yeast Display												16					
Future Use Collection		8[†]					8[†]		8	8		8			42		

∞ Prior to study procedures.

† Prior to study vaccination.

¬ Review/confirm information or activity in subjects previously consented and screened.

[1] Review results of clinical screening laboratory evaluations.

@ Complete medical history will be obtained by interview of subjects at the screening visit and will be updated on Day 1 prior to the first study vaccination and interim medical history will be obtained by interview of subjects at follow-up visits after the first study vaccination.

% Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

$ Vital signs assessed on Day 1 prior to the first study vaccination will be considered as baseline.

√ All current medications and medications taken within 60 days prior to signing the informed consent form.

^ Will be performed on all women of childbearing potential at screening (serum) and within 24 hours prior to study vaccination (urine) and results must be negative and known prior to each study vaccination.

~ Screening laboratories include: WBC, Hgb, HgbA1C, platelets, ANC, sodium, potassium, BUN (only assessed if creatinine is above the normal range) , creatinine, albumin, total protein, Alanine aminotransferase (ALT), hepatitis B surface antigen, hepatitis C antibody, HIV types 1 and 2, Prothrombin time (PT), partial thromboplastin time (PTT).

= All clinical screening laboratory evaluations are to be performed at screening and the values are to be reviewed prior to the first study vaccination on Day 1; no additional blood draws for this are required on Day 1.

& Retesting of values 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.

> If the initial laboratory screening occurred more than 28 days before baseline (Day 1) but the subject was unable to be vaccinated within the 28-day window (e.g., due to meeting Exclusion Criteria or for other reasons), the subject must have laboratories repeated.

{ } Targeted physical examination if indicated based on review of interim medical history.

[ ] ECG will be completed if clinically indicated based on signs/symptoms per the Investigator's judgement

* Safety laboratories include: WBC, Hgb, platelets, ANC, sodium, potassium, BUN (only assessed if creatinine is above the normal range), creatinine, Alanine aminotransferase (ALT).

[2] Refer to protocol for specific procedures and time points for ET or U/S visit.

[3] Single dose of 2 x 10^{11} vp ChAd3-EBO-Z.

[4] Single dose of Booster Vaccination (Group 1: placebo, Group 2: 2 x 10^{11} vp ChAd3-EBO-Z., Group 3: 1 x 10^{8} IU MVA-BN®-Filo).

[5] Volume of blood listed is in ml

APPENDIX B: ESTIMATED COMPARATIVE DAILY DOSES - INHALED CORTICOSTEROIDS FOR LONG-TERM ASTHMA CONTROL (12 YEARS AND OLDER)

Medication – Daily Dose	**Low**	**Medium**	**High**
Beclomethasone MDI	80 to 240 micrograms	more than 240 to 480 micrograms	more than 480 micrograms
40 micrograms per puff	1 to 3 puffs twice a day	4 to 6 puffs twice a day	
80 micrograms per puff	1 puff a.m., 2 puffs p.m.	2 to 3 puffs twice a day	4 or more puffs twice a day
Budesonide DPI	180 to 540 micrograms	more than 540 to 1,080 micrograms	more than 1,080 micrograms
90 micrograms per inhalation	1 to 3 inhalations twice a day		
180 micrograms per inhalation	1 inhalation a.m., 2 inhalations p.m.	2 to 3 inhalations twice a day	4 or more inhalations twice a day
Budesonide Nebules	not applicable	not applicable	not applicable
0.25 mg	not applicable	not applicable	not applicable
0.5 mg	not applicable	not applicable	not applicable
1.0 mg	not applicable	not applicable	not applicable
Ciclesonide MDI	160 to 320 micrograms	more than 320 to 640 micrograms	more than 640 micrograms
80 micrograms per puff	1 to 2 puffs twice a day	3 to 4 puffs twice a day	

160 micrograms per puff		2 puffs twice a day	3 or more puffs twice a day
Flunisolide MDI	320 micrograms	more than 320 to 640 micrograms	more than 640 micrograms
80 micrograms per puff	2 puffs twice a day	3 to 4 puffs twice a day	5 puffs or more twice a day
Fluticasone MDI	88 to 264 micrograms	more than 264 to 440 micrograms	more than 440 micrograms
44 micrograms per puff	1 to 3 puffs twice a day		
110 micrograms per puff		2 puffs twice a day	3 puffs twice a day
220 micrograms per puff		1 puff twice a day	2 or more puffs twice a day
Fluticasone DPI	100 to 300 micrograms	more than 300 to 500 micrograms	more than 500 micrograms
50 micrograms per inhalation	1 to 3 inhalations twice a day		
100 micrograms per inhalation		2 inhalations twice a day	3 or more inhalations twice a day
250 micrograms per inhalation		1 inhalations twice a day	2 or more inhalations twice a day
Mometasone DPI	110 to 220 micrograms	more than 220 to 440 micrograms	more than 440 micrograms
110 micrograms per inhalation	1 to 2 inhalations p.m.	3 to 4 inhalations p.m. or 2 inhalations twice a day	3 or more inhalations twice a day
220 micrograms per inhalation	1 inhalation p.m.	1 inhalation twice a day or 2 inhalations p.m.	3 or more inhalations divided in two doses

APPENDIX C: ACCEPTABLE RANGES OF SCREENING LABORATORY MEASUREMENTS

Lab Test Name	Clinical LaboratoryReference Range	Study Eligibility Acceptable Lower Limit	Study Eligibility Acceptable Upper Limit	Lab Unit
Hemoglobin, male	13.30 - 17.70	13.30	17.70	g/dL
Hemoglobin, female	11.70 - 15.70	11.70	15.70	g/dL
White blood cell count (WBC) (18 to <21 years)	4.5 – 13.0	3.6	13.0	K/mcL
White blood cell count(WBC) (≥21 years)	4.5 – 11.0	3.6	11.0	K/mcL
Absolute Neutrophil count*	1.80 – 7.70	1.80	7.70	K/mcL
Platelet count	135 - 466	135	466	K/mcL
PT	9.60 – 11.30	9.60	11.30	seconds
PTT	25.00 – 34.5	25.00	34.50	seconds
Sodium	136.0 – 145.0	136.0	145.0	mmol/L
Potassium	3.5 – 5.1	3.5	5.1	mmol/L
Blood urea nitrogen (BUN) †	9.0 – 23.00	<9.0†	23.00	mg/dL
Serum creatinine†, male	0.60 – 1.10	<0.60†	1.3	mg/dL

Serum creatinine†, female	0.50 – 0.80	<0.50†	1.2	mg/dL
Total protein	5.70 – 8.20	5.70	8.20	g/dL
Albumin	3.40 - 5.00	3.40	5.00	g/dL
Alanine transferase† (ALT)	≤ 49	n/a	49	unit/L
Hepatitis B surface antigen	Negative	Negative	Negative	n/a
Hepatitis C antibodies	Negative	Negative	Negative	n/a
HIV - 1/2 Ab	Negative	Negative	Negative	n/a
Serum hCG (females only)	Negative	Negative	Negative	n/a

* *ANC for subjects that are of African American and Middle Eastern descent may have values as low as* 0.8 K/mcL. *Subjects of this descent must have an ANC ≥ 0.8 K/mcL to be eligible to participate in the study if all other study criteria are met.*

† Values of *serum creatinine and blood urea nitrogen (BUN) below the lower limit of normal (LLN) are acceptable for study enrollment.*