# A Phase 1, Randomized, Parallel-Group, Double-Blind Trial of AV7909 (Liquid) and Thermostable AV7909 (Lyophilized) in Healthy Adult Volunteers

**DMID Protocol Number:** 17-0093

**DMID Funding Mechanism:** Vaccine and Treatment Evaluation Units

**Pharmaceutical Support:**
Emergent BioSolutions

**IND Sponsor:** Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health

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**Version:** 4.0

11 July 2022

# STATEMENT OF COMPLIANCE

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

- United States (US) 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), 21 CFR 812 (Investigational Device Exemptions)
- International Council for Harmonisation (ICH) E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) Guidance for Industry, published in the Federal Register (83 Federal Register 8882 (2018)) 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 current ICH E6 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)/Institutional Ethics Committee (IEC) approval, except when necessary to protect the safety, rights, or welfare of subjects.

Site Principal Investigator:

Signed: _____ Date: _____

Name: James D. Campbell, MD, MS
Title: Principal Investigator

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| AE | Adverse Event/Adverse Experience |
| AESIs | Adverse Events of Special Interest |
| ALT | Alanine Aminotransferase |
| AVA | Anthrax Vaccine Adsorbed |
| AV7909 | Anthrax Vaccine with CPG7909 Adjuvant |
| β hCG | Beta- human Chorionic Gonadotropin (Pregnancy Test) |
| BMI | Body Mass Index |
| BP | Blood Pressure |
| BUN | Blood Urea Nitrogen |
| CFR | Code of Federal Regulations |
| CI | Confidence Interval |
| CMS | Clinical Materials Services |
| COI | Conflict of Interest |
| CPG 7909 | a CPG oligodeoxynucleotide adjuvant |
| CpG55.2 | a CPG oligodeoxynucleotide adjuvant |
| Cr | Creatinine |
| CRP | C-Reactive Protein |
| CROMS | Clinical Research Operations and Management Support |
| CSR | Clinical Study Report |
| D | Day(s) |
| DNA | Deoxyribonucleic Acid |
| DCF | Data Collection Form |
| DMID | Division of Microbiology and Infectious Diseases, NIAID, NIH |
| ECG | Electrocardiogram |
| eCRF | Electronic Case Report Form |
| eClinical | Electronic Data Capture System |
| eMemory Aid | Electronic Memory Aid |
| ELISA | Enzyme Linked ImmunoSorbent Assay |
| FDA | Food and Drug Administration |
| FWA | Federal-Wide Assurance |
| GCP | Good Clinical Practice |
| GMT | Geometric Mean Titer |
| HBsAg | Hepatitis B surface Antigen |
| HCV | Hepatitis C Virus |
| HEENT | Physical Exam of the Head, Eyes, Ears, Nose, and Throat |
| Hgb | Hemoglobin |
| HIPAA | Health Insurance Portability and Accountability Act |

| | |
|---|---|
| HIV | Human Immunodeficiency Virus |
| HLGT | High Level Group Term |
| HRPO | Human Research Protections Office |
| IATA | International Air Transport Association |
| ICF | Informed Consent Form |
| ICH | International Council for Harmonisation |
| IgG | Immunoglobulin G |
| IM | Intramuscular(ly) |
| IND | Investigational New Drug |
| IRB | Institutional Review Board |
| ISM | Independent Safety Monitor |
| IU/L | International Unit(s) per Liter |
| LLOQ | Lower Limit of Quantitation |
| MAAE | Medically-Attended Adverse Event |
| MedDRA® | Medical Dictionary for Regulatory Activities |
| mg/dL | Milligrams per Deciliter |
| mITT | Modified Intent-to-Treat |
| mL | Milliliter(s) |
| $mm^3$ | Cubic Millimeter(s) |
| MOP | Manual of Procedures |
| N | Number of Subjects |
| NIAID | National Institute of Allergy and Infectious Diseases, NIH, DHHS |
| NIH | National Institutes of Health |
| OAC | Office of Accountability and Compliance |
| ODNs | Synthetic oligodeoxynucleotides |
| OER | Office of Extramural Research |
| OHRP | Office for Human Research Protections |
| PHI | Personal (Protected) Health Information |
| PA | Protective Antigen |
| PI | Principal Investigator |
| PII | Personally Identifiable Information |
| PIMMCs | Potentially Immune-Mediated Medical Conditions |
| PP | Per Protocol |
| QA | Quality Assurance |
| QC | Quality Control |
| RF | Rheumatoid Factor |
| SAE | Serious Adverse Event/Serious Adverse Experience |
| SAP | Statistical Analysis Plan |
| SDCC | Statistical and Data Coordinating Center |

| | |
|---|---|
| SIRVA | Shoulder Injury Related to Vaccine Administration |
| SMC | Safety Monitoring Committee |
| SOP | Standard Operating Procedure |
| TNA | Toxin Neutralization Assay |
| ULN | Upper Limit of Normal |
| UMB | University of Maryland, Baltimore |
| US | United States |
| V | Visit(s) |
| VTEU | Vaccine and Treatment Evaluation Unit |
| WHO | World Health Organization |

# PROTOCOL SUMMARY

| | |
|---|---|
| **Title:** | A Phase 1, Randomized, Parallel-Group, Double-Blind Trial of AV7909 (Liquid) and Thermostable AV7909 (Lyophilized) in Healthy Adult Volunteers |
| **Phase:** | I |
| **Population:** | 40 males and non-pregnant females, 18 to 45 years of age, inclusive, who are in good health and meet all eligibility criteria |
| **Number of Sites:** | 1 Vaccine and Treatment Evaluation Unit (VTEU) |
| **Study Duration:** | Approximately 16 months from first screening visit for first participant until last follow-up visit for last participant |
| **Subject Participation Duration:** | Approximately 13 months |
| **Estimated Time to Complete Enrollment:** | Approximately6 months |
| **Description of Agents:** | • AV7909 liquid formulation vaccine<br>• AV7909 lyophilized formulation vaccine |

## Table 1: Study Objectives and Outcome Measures

| **Primary Objective: Safety** | **Primary Safety Outcome Measures** |
|---|---|
| • To assess the safety of lyophilized and liquid formulations of AV7909. | • Occurrence of all SAEs through approximately 12 months following receipt of the second study vaccination (approximately Day 380).<br>• Occurrence of abnormal clinical safety laboratory AEs at approximately Day 29.<br>• Occurrence of all protocol specified AESIs (i.e., PIMMCs) and MAAEs, from the time of the first study vaccination through approximately 12 months following the second vaccination (approximately Day 380).<br>• Occurrence of unsolicited non-serious adverse events from the time of the first study vaccination through approximately Day 64. |
| **Primary Objective: Tolerability** | **Primary Tolerability Outcome Measures** |
| • To assess the tolerability of lyophilized and liquid formulations of AV7909. | • Occurrence of solicited injection site and systemic reactogenicity events in the week after each study vaccination (Days 1 through 8 and Days 15 through 22, inclusive). |
| **Secondary Objective: Immunogenicity** | **Secondary Immunogenicity Outcome Measures** |
| • To obtain initial estimate of comparative immunogenicity of liquid and lyophilized formulations of AV7909. | • Geometric mean titer and 95% confidence intervals of antibodies assayed by toxin neutralization assay ($ED_{50}$ and $NF_{50}$) and anti-PA IgG by ELISA in each study group before vaccination and on approximately Days 8, 15, 22, 29, 64, 195, and 380.<br>• Proportion and 95% confidence intervals of participants in each study group with seroconversion for antibodies detected by TNA and ELISA (defined as a ≥4-fold increase over baseline levels, or a ≥4-fold increase over the lower limit of quantification [LLOQ] if the baseline value is < LLOQ) on approximately Days 8, 15, 22, 29, 64, 195, and 380.<br>• Proportion and 95% confidence intervals of participants in each study group with putative seroprotection (defined as $NF_{50} \geq 0.56$) before vaccination and on approximately Days 8, 15, 22, 29, 64, 195, and 380. |
| **Exploratory Immunogenicity Objectives** | **Exploratory Immunogenicity Study Outcomes** |
| • None | • None |

**Description of Study Design:**

The study is a phase I randomized, parallel-group, double-blind controlled interventional trial in which the safety, tolerability, and immunogenicity of 2 formulations of adjuvanted anthrax vaccine (AV7909), lyophilized and liquid, both manufactured by Emergent BioSolutions, will be assessed and compared. AV7909 is an investigational vaccine that combines the currently licensed Anthrax Vaccine Adsorbed (AVA, BioThrax) with the adjuvant CPG 7909, an oligonucleotide. Forty healthy young adults, 18 to 45 years old, inclusive, who meet all eligibility criteria, will be randomly allocated to one of two study groups in a 1:1 ratio: 20 will receive AV7909 as the thermostable lyophilized product and 20 will receive AV7909 as the liquid product. Stratification by age category and by gender will assure that near equal numbers of younger (18-30 years) and older (31-45 years) males and females are assigned to each vaccine. The vaccines will be given intramuscularly in a 2-dose schedule, 2 weeks apart. Data entry will be "direct", that is, without the significant use of paper source documents or case report forms. Participants will be followed for safety and tolerability by in-person visits, electronic reactogenicity records (eMemory Aids), and safety laboratory values. A Safety Monitoring Committee will evaluate the safety data if halting rules are met or if deemed necessary by the sponsor or principal investigator. Immunogenicity will be evaluated by pre-vaccination and multiple post-vaccination assessments of toxin-neutralizing antibodies and IgG anti-PA antibodies assayed by Enzyme Linked ImmunoSorbent Assay (ELISA) over approximately one year. Participants will be followed for 1 year following the second dose of vaccine.

Safety will be assessed by evaluation of laboratory values, non-serious unsolicited Adverse Events, Serious Adverse Events (SAEs), Medically Attended Adverse Events (MAAEs), and Adverse Events of Special Interest (AESIs), which, for this protocol, are defined as Potentially Immune-Mediated Medical Conditions (PIMMCs). Unsolicited non-serious adverse events (AEs) will be collected from Day 1 through approximately Day 64, inclusive. SAEs, MAAEs, and AESIs (PIMMCs) will be collected from the time of study vaccination through approximately Day 380. Clinical safety laboratory evaluations will be performed at screening and at approximately Day 29

Reactogenicity will be measured by the occurrence of solicited injection site and systemic reactions in the week after each study vaccination (Days 1 through 8 and target of Days 15 through 22, inclusive). Any reactogenicity events that persist on Day 8 or Day 22 will be followed until resolution. Only pharmacy personnel, the SDCC unblinded team, and an unblinded vaccinator and checker will have access to the study codes; all other study personnel and the participants will be blinded.

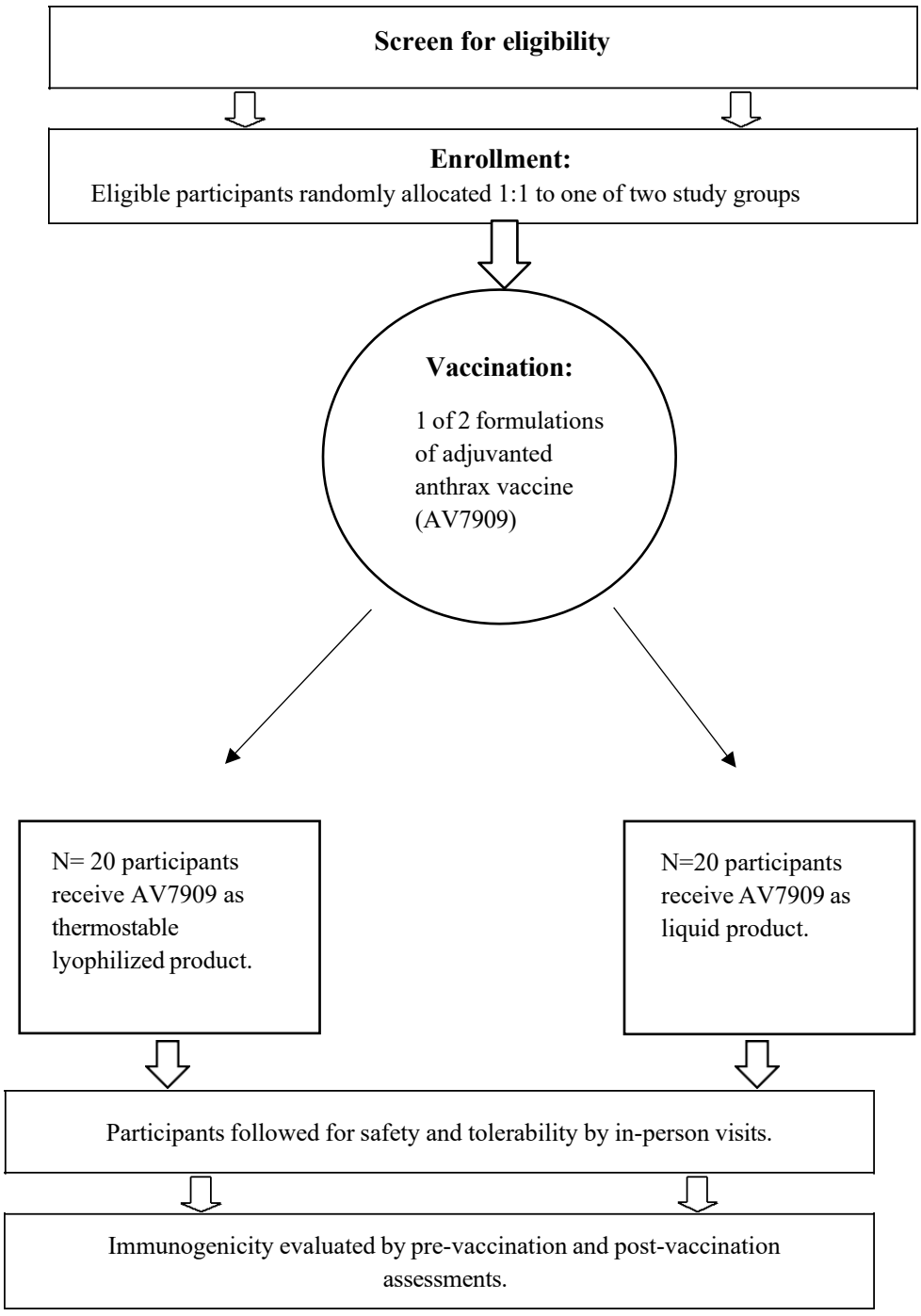
Immunogenicity testing will include performing serological assays to assess toxin neutralizing antibodies (reported as $ED_{50}$ and $NF_{50}$) and IgG anti-PA antibodies assayed by Enzyme Linked ImmunoSorbent Assay (ELISA) prior to

vaccination (on Day 1) and on approximately Days 8, 15, 22, 29, 64, 195, and 380.

## Table 2: Study Groups

| | | Day 1 | Day 15 |
|---|---|---|---|
| **Study Group** | **N** | **Study Vaccine** | **Study Vaccine** |
| **1** | 20 | AV7909 liquid | AV7909 liquid |
| **2** | 20 | AV7909 lyophilized | AV7909 lyophilized |
| **Total** | **40** | | |

## Figure 1: Schematic of Study Design



# 1 KEY ROLES

| | |
|---|---|
| **Principal Investigator:** | James D. Campbell, MD, MS<br>University of Maryland School of Medicine<br>Center for Vaccine Development<br>Baltimore, MD 21201 |
| **DMID Clinical Project Manager:** | Onyinye Erondu, RN, MS<br>Division of Microbiology and Infectious Diseases<br>NIAID, NIH |
| **DMID Medical Monitor:** | Jorge Mejia-Galvis, MD, MBA<br>Division of Microbiology and Infectious Diseases<br>NIAID, NIH |
| **DMID Medical Officer:** | Maryam Keshtkar-Jahromi, MD, MPH<br>Division of Microbiology and Infectious Diseases<br>NIAID, NIH |
| **DMID Scientific Lead:** | Colleen Sico, ChemE MBA<br>Division of Microbiology and Infectious Diseases<br>NIAID, NIH |
| **Safety and Pharmacovigilance:** | DMID Pharmacovigilance Group<br>Clinical Research Operations and Management Support (CROMS) |
| **Site Principal Investigators:** | Not applicable (This is a single site study.) |
| **Statistical and Data Coordinating Center:** | The Emmes Company, LLC |
| **Clinical Materials Services:** | Fisher BioServices |
| **Central (Clinical) Laboratory:** | Garcia Laboratory |

| | |
|---|---|
| **Anthrax Toxin Neutralization and ELISA Serological Assays Laboratory:** | Battelle |

# 2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

## 2.1 Background Information

*Bacillus anthracis* is an aerobic, spore-forming, gram-positive bacterium that causes the disease anthrax in animals and people. Zoonotic anthrax still occurs in many parts of the world but is completely controlled in the United States as a result of animal vaccination programs and other measures, although sporadic cases of anthrax are reported after exposure to imported animal hides.[1] Unintentional human disease usually occurs after contact with infected animals or animal products. Routes of transmission and types of disease are cutaneous, injection drug use associated, gastrointestinal, and inhalational. Inhalational anthrax may occur when spores become airborne as a result of certain industrial processes, as a result of laboratory accidents, and due to intentional exposure related to biological terrorism or biological warfare.[2]

Anthrax has previously been used as a biological agent of terror.[3] In 2001, 22 people were exposed to anthrax spores distributed through the US Postal Service and 5 of the cases were fatal. This event caused widespread fear and panic. Even today, it remains one of the most likely agents to be used in an act of bioterror because it is easy to cultivate, easy to weaponize, easy to distribute, and has a high case fatality rate.[2]

The anthrax vaccine that is currently licensed in the US, Anthrax Vaccine Adsorbed (AVA, BioThrax™) is produced from the culture filtrate of an attenuated, toxigenic strain of *B. anthracis*, and contains Protective Antigen (PA, the binding domain of the anthrax toxin), small amounts of other anthrax bacterial products, the adjuvant aluminum hydroxide (Alhydrogel), the stabilizer formaldehyde, and the preservative benzethonium chloride. This vaccine is highly protective against inhalational *B. anthracis* spore challenge in rabbits and monkeys [4]. A similar vaccine had 92% efficacy against clinical anthrax among workers in New England wool mills in the middle of the last century,[5] but no other human efficacy trials have ever been performed. The vaccine is recommended as pre-exposure prophylaxis for members of the US Armed Forces, for some laboratory workers, some “first responders”, and for others who may be occupationally exposed.[6] It has also been offered, and is recommended, for cases of post-exposure in conjunction with antibiotics.[6] It has not been studied in children.

## 2.2 Scientific Rationale

The anthrax vaccine that is currently licensed in the US, AVA, has been shown to be safe and immunogenic in people and has been shown to lead to antibody responses that, in non-human primate models of lethal anthrax spore challenge, are protective, even in the absence of

antibiotics[4]. However, the vaccine was first given subcutaneously, leading to significant local reactogenicity, and it requires 3 priming doses and an onerous schedule for recipients to remain immune. An improved vaccine that leads to faster immune responses and requires fewer doses, while remaining safe and well tolerated, would be welcomed.

Due to the large number of doses required to generate a primary immune response in humans that leads to putative protective levels, as evidenced by animal challenge model protection, various approaches have been taken to improve anthrax vaccines. One approach that has proven successful to date is the addition of the TLR9 agonist adjuvant CPG 7909 to the currently licensed vaccine. This new vaccine, named AV7909, is being developed by Emergent BioSolutions as a vaccine with an indication for postexposure prophylaxis (PEP) that is intended to decrease the risk of anthrax disease after exposure. AV7909 has been shown to be safe and has improved immunogenicity when compared to AVA.[7-9] Early studies have shown that the addition of CPG 7909 to AVA has allowed for intramuscular injection, a priming series of only 2 doses, and resulted in more rapid induction of putatively protective antibody responses. AV7909 has been studied in animals and humans with a safety record similar to AVA itself. This vaccine, once studied in increasing numbers of persons, may prove superior enough to AVA to allow it to replace AVA as the standard of care. However, the current formulation is liquid and therefore prone to a shorter shelf life and stricter temperature storage requirements. Since anthrax vaccine sufficient for a response to an intentional act of bioterror is stored in the National Strategic Stockpile, a thermostable (lyophilized) formulation of the AV7909 adjuvanted anthrax vaccine would offer many advantages to a liquid formulation.

The proposed trial will assess, in healthy adults, the safety, reactogenicity, and immunogenicity of the thermostable lyophilized formulation of AV7909 compared to the liquid formation of AV7909.

## 2.3 Potential Risks and Benefits

### 2.3.1 Potential Risks

The potential risks of participating in this trial are those that may occur due to blood drawing, the IM injection itself, reactions to the vaccines, and breach of confidentiality.

Phlebotomy Risks. Drawing blood is typically well tolerated and uneventful. It often causes transient mild discomfort. Approximately 2% of persons who have blood drawn will experience “near syncope”, in which they feel lightheaded or have other symptoms, but do not lose consciousness. Syncope (fainting) is a transient autonomic (vasovagal) response to phlebotomy that occurs in approximately 1% of blood donors and fewer than 1% of those having diagnostic phlebotomy.[10] It is managed by having the participant lie down, loosening his or her clothes,

monitoring vital signs, reassuring the participant, and giving fluids. Minor bruising at the blood draw site may occur in up to 12% and a hematoma in 2-3%. These events can be prevented or decreased in frequency by employing experienced phlebotomists and can be ameliorated, when they occur, by applying pressure to the draw site for several minutes. Rarely, following phlebotomy, persons may have nerve injury (typically lateral antebrachial nerve) causing pain, paresthesia, and motor or sensory loss. This phenomenon is uncommon and typically resolves quickly with watchful waiting only.[11] Infection at the site of the blood draw is exceedingly rare and can be avoided by using antiseptics and other appropriate techniques. In a study of over 4000 persons undergoing routine venipuncture for insurance applications, there were no serious local reactions such as cellulitis or phlebitis.[10]

Intramuscular Injection Risks. The injection of a study product into the deltoid muscle, as will be done in this study, is typically well tolerated. However, it often causes transient discomfort. Intramuscular injections may cause near syncope or syncope, although less commonly than phlebotomy. It can also cause an abscess, a hematoma, injury to blood vessels and peripheral nerves, or tingling or numbness. Injection too proximally on the lateral aspect of the deltoid can lead to injury of the bursa, which can cause Shoulder Injury Related to Vaccine Administration, or SIRVA.[12] Proper education and technique reduce the risk of adverse events after IM injection.

Vaccine Administration Risks. The risk profile for AVA (BioThrax) is well known, given that millions of persons have been vaccinated. The risks of AV7909 are less well characterized, given that fewer persons have been vaccinated with this product.

Risks of AVA (BioThrax). Since the licensure of BioThrax in 1970, more than 3.3 million individuals, primarily military personnel, have been vaccinated in the pre-exposure setting. The safety profile of BioThrax and its associated risks are summarized in the BioThrax prescribing information. The most common (≥ 10%) local (injection-site) adverse reactions observed in clinical studies are tenderness, pain, erythema, edema, and arm motion limitation. The most common (≥ 5%) systemic adverse reactions are muscle aches, headache, and fatigue.

Risks of CPG 7909. CPG 7909 has been investigated clinically since the mid-1990s for indications that have included cancer monotherapy, combination use with anti-cancer therapies, and vaccine adjuvant against infectious diseases and cancers. Potential risks associated with AV7909 include hypersensitivity reactions, autoimmune reactions, and more minor local and systemic reactogenicity effects. Other oligonucleotides have been evaluated as vaccine adjuvants, including CPG1018, the adjuvant in Heplisav-B, a hepatitis B vaccine now licensed in the US for persons 18 years and older.

Risks of AV7909. Emergent BioSolutions has sponsored three completed clinical studies in healthy participants 18 through 50 years of age as part of the AV7909 development program: a phase 1, a phase 1/2, and a phase 2 trial. [7,9,13] A total of 241 participants have been exposed to a

combination of AVA and CPG7909. In the initial trial, a formulation with AVA + 1.0 mg CPG 7909 was tested and was well tolerated with a trend toward increased frequency and severity of local and systemic reactions in the AV7909 formulation compared to BioThrax.[9] The second trial identified a formulation of AV7909 with a lower CPG7909 concentration (0.25 mg CPG 7909) for further clinical development that produced an enhanced immune response without increased reactogenicity. [7] This formulation was evaluated in the phase 2 trial described below and is the formulation to be tested in the current study.

In 2014, a phase 2, randomized, double-blinded, BioThrax-controlled study was conducted to evaluate the safety and immunogenicity of 3 IM vaccination schedules and 2 dose levels of AV7909 in 168 healthy adults.[13] The results of this study found that AV7909 was well tolerated: 4.2% of subjects discontinued vaccination due to AEs; no serious adverse events (SAEs) were reported that were potentially vaccine related; no reported AEs of potential autoimmune etiology were reported. The vaccine produced a more rapid immune response compared to BioThrax as measured by TNA $NF_{50}$ values≥0.56 (a putative correlate of protection) obtained through Day 63.

Hypersensitivity and Anaphylactic/Anaphylactoid Reactions. Parenteral administration of any protein product may be associated with immediate-type hypersensitivity reactions that can manifest as urticaria, shortness of breath, wheezing, nausea and cramping, and in severe cases, hypotension. Acute allergic reactions, including anaphylaxis, have occurred with BioThrax. Reports of hypersensitivity, urticaria, and pruritus have rarely occurred following administration of AV7909. Appropriate medical treatment and supervision will be available to manage possible anaphylactic reactions following administration of the vaccine and the vaccine will not be administered to participants with known sensitivity to any of the vaccine components, e.g., synthetic oligodeoxynucleotides (ODNs), formaldehyde, benzethonium chloride (phemerol), or aluminum. The vaccine stoppers do not contain latex.

Potentially Immune Mediated Medical Conditions (PIMMCs). There is a plausible potential for CPG ODN adjuvants to trigger autoimmune disease in susceptible individuals [14], possibly as a result of non-specific activation of T or B lymphocytes. No participants in completed AV7909 clinical trials have reported AEs related to autoimmune disorders. Additionally, no AEs suggestive of autoimmune disease have been reported in the reviewed published data on the CPG 7909-adjuvanted infectious disease vaccine trials. A number of these trials reported participants with moderate, transient elevations above the normal range of anti-double stranded deoxyribonucleic acid (anti-dsDNA) antibody values or positive antinuclear antibody (ANA) or mild elevations in rheumatoid factor (RF) following vaccination, which typically returned to normal by the next dose or by the end of the study and were not associated with clinical symptoms.[15-18] In a phase 3 trial of Heplisav-B, a recently FDA-licensed vaccine that contains a different CPG ODN, CPG 1018, in participants followed for 13 months after the first dose of vaccine, the incidences of new onset immune-mediated AEs (as reviewed by independent adjudicators) were not clinically different

between the Heplisav-B group (0.1%; 4/5587) and the non-adjuvanted Engerix-B group (0%; n=2781), nor were these AEs in the Heplisav-B group considered related to vaccination.

In oncology trials of CPG 7909, which typically have used higher doses and treatment durations than expected with AV7909 vaccine exposure, autoimmune disease has been reported in multiple participants, although causal attribution to the study product has been complicated by comorbidities and concomitant products/therapies. The autoimmune conditions observed have included polyarthralgia, arthritis, Sjögren's Syndrome, autoimmune thyroiditis, vitiligo, Guillain-Barré syndrome, and ulcerative colitis.

Because of this potential risk, individuals with a history of autoimmune disorders or active autoimmune disease (PIMMCs) will be ineligible for this trial. Additionally, because of the possibility of a delay between the vaccination and onset of symptoms indicative of a disease process of this kind, all participants will be followed for PIMMCs throughout the trial (for 12 months after the second vaccination, to approximately Day 380). PIMMCs will be captured as "Adverse Events of Special Interest" (AESI). Blood samples will be collected before vaccination from all participants for possible laboratory evaluation should suspicion of a PIMMC arise during study participation. Participants who discontinue study treatment will be encouraged to attend all subsequent clinic visits and safety follow-up contacts to facilitate this monitoring.

Local Reactogenicity. During clinical testing in which 241 participants were exposed to the combination of BioThrax + CPG 7909, the most common local reactions were mild to moderate pain, tenderness, and arm motion limitation. These reactions often resolved within 48 hours after vaccination. The most common local AEs (reported in ≥ 5% of participants in at least one of the AV7909 arm(s) from the three completed clinical trials) included injection site pain, arm movement impairment, warmth, edema/induration, erythema, hemorrhage, and pruritus.

Systemic Reactogenicity. During clinical testing to date, in completed trials in which participants were exposed to the combination of BioThrax + CPG 7909, systemic reactogenicity manifested primarily as mild to moderate fatigue, muscle ache, and headache. The most common systemic AEs (reported in ≥ 5% of participants in at least one of the AV7909 arm(s) from the three completed clinical trials) included fatigue, myalgia, headache, respiratory rate increased, pyrexia, pharyngolaryngeal pain, nausea, upper respiratory tract infection, and chills.

Laboratory Findings. During clinical testing to date, in completed trials in which participants were exposed to the combination of BioThrax + CPG 7909, the most common reported laboratory findings after vaccination were hypokalemia, lymphocyte count decreased, leukopenia, hypoglycemia, blood creatine phosphokinase increased, and hemoglobin decreased.

Pregnancy and Lactation. No reproductive toxicity studies have been performed with AV7909. BioThrax can cause fetal harm when administered to a pregnant woman. CPG 7909 has been

determined to be embryolethal in rabbits and teratogenic in developing rats and rabbits at doses far in excess of the individual vaccination dose for AV7909 (0.25 mg). In rats, fetal skeletal anomalies were noted when administered at a dose of > 3 mg/kg/day; in rabbits, slightly reduced numbers of viable fetuses were noted when administered at a dose of 3 mg/kg/day, and external, visceral, and skeletal malformations occurred when administered at >1 mg/kg/day.

Given these findings, breastfeeding or a positive pregnancy test will preclude study enrollment. All women of childbearing potential will have a negative pregnancy test before each vaccine administration and will agree to use effective contraception from at least one month prior to Day 1 and continued through Day 75 (60 days after second vaccination). Confirmed pregnancy will result in discontinuation of vaccinations. Participants who become inadvertently pregnant during the trial will be monitored for pregnancy outcomes, if the participant provides permission, according to protocol-specified procedures.

Risk of loss of privacy or confidentiality. Participants will be asked to provide, in a private setting, Personally Identifying Information (PII) and Protected Health Information (PHI). All attempts will be made to keep PII and PHI confidential within the limits of the law. However, there is a chance that unauthorized persons will gain access to a participant's PII or PHI. All hard copy research records will be kept in a locked file cabinet or maintained in a locked room at the participating VTEU site. Electronic files and access to the central clinical and laboratory websites at which study-related data are stored will be password protected. Only people who are involved in the conduct, oversight, monitoring, or auditing of this study will be allowed access to the confidential information that is collected. Any publications from this study will not use information that will identify participants by name. Organizations that may inspect and/or copy research records maintained at the participating VTEU site for quality assurance and data analysis include groups such as National Institute of Allergy and Infectious Diseases (NIAID) and the Food and Drug Administration (FDA) and their contractors. They also include the Human Research Protections Office (HRPO) at the University of Maryland, Baltimore (UMB) and its Institutional Review Board (IRB), and the Office of Accountability and Compliance (OAC) at UMB.

Unknown risks. There may be unknown risks, discomforts, or side effects.

### 2.3.2 Known Potential Benefits

Participants in this study may have no personal, direct benefits from participation. Although they are not currently identified as a person at risk for contracting anthrax, participants may develop immune responses to the anthrax vaccines being studied in this protocol that are protective against anthrax or that may mitigate anthrax disease following exposure. Even without any personal direct benefits, participants may contribute to a societal benefit through the

improvement of our understanding of these vaccines and by contributing to the protection of persons at risk for or exposed to anthrax.

# 3 STUDY OBJECTIVES AND OUTCOME MEASURES

Refer to Table 1: Study Objectives and Outcome Measures

# 4 STUDY DESIGN

The study is a phase I randomized, parallel-group, double-blind controlled interventional trial in which the safety, tolerability, and immunogenicity of 2 formulations of adjuvanted anthrax vaccine (AV7909), lyophilized and liquid, both manufactured by Emergent BioSolutions, will be assessed and compared. AV7909 is an investigational vaccine that combines the currently licensed Anthrax Vaccine Adsorbed (AVA, BioThrax) with the adjuvant CPG 7909, an oligonucleotide. Forty healthy young adults, 18 to 45 years old, inclusive, who meet all eligibility criteria, will be randomly allocated to one of two study groups in a 1:1 ratio: 20 will receive AV7909 as the thermostable lyophilized product and 20 will receive AV7909 as the liquid product. Stratification by age category and by gender will assure that near equal numbers of younger (18-30 years) and older (31-45 years) males and females will be assigned to each vaccine. The vaccines will be given intramuscularly in a 2-dose schedule, 2 weeks apart. Data entry will be "direct", that is, without the significant use of paper source documents. Participants will be followed for safety and tolerability by in-person visits, electronic reactogenicity records (eMemory Aids), and safety laboratory values. A Safety Monitoring Committee will evaluate the safety data whenever necessary (as determined by the sponsor or principal investigator) and in the event of a halting rule occurring. Immunogenicity will be evaluated by pre-vaccination and multiple post-vaccination assessments of toxin-neutralizing antibodies as determined by $ED_{50}$ and $NF_{50}$ and by IgG antibodies against PA measured by ELISA. Participants will be followed for 1 year following the second dose (through approximately Day 380).

Safety will be assessed by evaluation of non-serious unsolicited Adverse Events, Serious Adverse Events (SAEs), Medically Attended Adverse Events (MAAEs), Adverse Events of Special Interest (AESIs) [the AESIs collected in this study are Potentially Immune-Mediated Medical Conditions (PIMMCs)], and by laboratory evaluations. SAEs, MAAEs, and AESIs will be collected from the time of study vaccination through approximately Day 380. Non-serious unsolicited AEs will be collected from Day 1 to Day 64, inclusive. Clinical laboratory evaluations for eligibility will be the following: specific hematology parameters, chemistry parameters, , specific urine tests for drugs of abuse, and hemoglobin $A_1C$. Also, an electrocardiogram will be performed at screening and reviewed by an investigator and cardiologist. The cardiologist will determine if any findings on the electrocardiogram are significant and thereby exclusionary. At approximately Day 29, laboratory tests for hematology and chemistries will be repeated.

Reactogenicity will be measured by the occurrence of solicited injection site and systemic reactions in the week after each study vaccination (Days 1 through 8 and target of Days 15 through 22, inclusive). The window for dose 2 includes Days 15 to 18. For participants who are vaccinated beyond Day 15, the study schedule will adjust so that a full week of reactogenicity data are collected, regardless. Any of these events that persist on Day 8 or Day 22 (or the final

day of the second reactogenicity period) will be followed until resolution. Only pharmacy personnel, the Emmes unblinded team, and an unblinded vaccinator and checker will have access to the study codes; all other study personnel and all participants will be blinded.

Immunogenicity testing will include performing serological assays to assess for toxin neutralizing antibodies (reported as $ED_{50}$ and $NF_{50}$), the gold standard assay for assessing response and protection following anthrax vaccines, prior to vaccination and on approximately Days 8, 15, 22, 29, 64, 195, and 380. Should the second dose of vaccine be given after Day 15 (the acceptable window is Days 15 to 18, inclusive) the remainder of the study schedule days shift accordingly, to maintain the intervals in the schedule. In addition, anti-PA IgG antibodies will be measured by ELISA from the serum of participants, on those same days.

For additional details on study procedures and evaluations and the study schedule by study visits/days, see Sections 7 and 8 as well as Appendix A: Schedule of Study Procedures and Evaluations and Appendix B: Adverse Events of Special Interest.

# 5 STUDY ENROLLMENT AND WITHDRAWAL

Forty (40) males and non-pregnant females, 18 to 45 years of age, inclusive, who are in good health and meet all eligibility criteria will be enrolled at one VTEU site. The target population will reflect the community at large at the participating VTEU site, but in order to assure enrollment of near equal numbers in each study group (AV7909 Liquid and AV7909 Lyophilized) by sex and age category, a stratified enrollment procedure will be employed. The strata used will be males/females and age 18-30 years/age 31-45 years.

The estimated time to complete enrollment in this trial, from first participant enrolled until last participant enrolled, is approximately 3 months. Information regarding this trial may be provided to potential participants who have previously participated in vaccine trials, other than anthrax vaccine trials, conducted at the VTEU site. Other forms and/or mechanisms of recruitment may also be used. The IRB will approve the recruitment process and all materials prior to use.

Participant inclusion and exclusion criteria will be assessed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator (PI) or sub-investigator.

No exemptions are granted on participant inclusion or exclusion criteria in DMID-sponsored studies. Questions about eligibility will be directed toward the DMID Medical Officer for this trial.

## 5.1 Eligibility Criteria

### 5.1.1 Participant Inclusion Criteria

Participants eligible to participate in this trial must meet all the following inclusion criteria:

1. Provide written informed consent prior to initiation of any study procedures.
2. Understand and comply with planned study procedures, including completion of the electronic memory aid, and be available for all study visits.
3. Agree to the collection of venous blood, per protocol.
4. Have adequate venous access for phlebotomies.
5. Be a male or non-pregnant female, 18 to 45 years of age, inclusive, at the time of enrollment.

6. Be in good health[1].

*[1]As determined by medical history and physical examination to evaluate acute or currently ongoing chronic medical diagnoses or conditions, which would affect the assessment of the safety of participants or the immunogenicity of study vaccinations. Chronic medical diagnoses or conditions, defined as those that have been present for at least 90 days, should be stable (not worsening) for the last 60 days (no hospitalizations, emergency room or urgent care for condition, or invasive medical procedure and no adverse symptoms that need medical intervention such as medication change indicative of worsening/supplemental oxygen). This includes no change in chronic prescription medication, dose or frequency, indicative of worsening disease, 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, will not be considered a deviation of this inclusion criterion. Participants may be on chronic or as needed (prn) medications if, in the opinion of the site PI or appropriate sub-investigator, they pose no additional risk to participant safety or assessment of reactogenicity and immunogenicity and do not indicate a worsening or treatment of continued symptoms of medical diagnosis or condition. Herbals, vitamins, and supplements are permitted.*

7. Have an oral temperature less than 100.0°F.

8. Have a pulse 51 to 100 beats per minute, inclusive.

9. Have a systolic blood pressure 85 to 140 mmHg, inclusive.

10. Have a diastolic blood pressure 55 to 90 mmHg, inclusive.

11. Have a calculated body mass index (BMI) less than or equal to 35.0 $kg/m^2$ at screening.

12. Screening laboratories[2] in table below are within acceptable parameters.

2

| **Test** | **Eligibility Criterion** |
|---|---|
| BUN | <23 mg/dL |
| Serum creatinine (female) | <1.3 mg/dL |
| Serum creatinine (male) | < 1.4 mg/dL |
| Alkaline phosphatase (female) | <147 U/L |
| Alkaline phosphatase (male) | <192 U/L |
| ALT (aka SGPT) | <68 U/L |
| Total bilirubin | < 1.3 mg/dL |
| Hemoglobin (female) | >10.9 g/dL |
| Hemoglobin (male) | > 12.4 g/dL |
| White blood cell count | 3000-12,000 cells/$mm^3$ |
| Absolute eosinophil count | <1201 cells/$mm^3$ |
| Absolute neutrophil count | >1200 cells/$mm^3$ |
| Platelets | >126,000 cells/$mm^3$ |
| Hemoglobin $A_1C$ | <6.5% |

| | |
|---|---|
| Urine for Drugs of Abuse* | All negative |
| HBsAg | Non-reactive |
| HCV antibodies | Negative |
| HIV 4th generation test | Negative |

**amphetamines, barbiturates, benzodiazepines, cocaine, methadone, opiates, oxycodone/oxymorphone, phencyclidine (PCP), and propoxyphene. Note that a prospective participant with a positive urine for drugs of abuse test for a medication reported by the participant and used to treat a condition that does not make them otherwise ineligible, may be permitted to enroll, at the discretion of the investigator or his designee.*

13. Have no clinically significant findings[3] on 12-lead electrocardiogram.

*[3]Clinical significance will be determined by a cardiologist. Examples of findings that will lead to exclusion are significant left ventricular hypertrophy, right or left bundle branch block, advanced A-V heart block, non-sinus rhythm (excluding isolated premature atrial contractions), pathologic Q wave abnormalities, significant ST-T wave changes, prolonged QTc interval.*

14. Heterosexually active females of childbearing potential[4] must use an acceptable contraception method[5] from at least 30 days before the first until 60 days after the second study vaccination.

*[4]Not sterilized via bilateral oophorectomy, salpingectomy, hysterectomy, or successful Essure® placement (permanent, non-surgical, non-hormonal sterilization) with documented radiological confirmation test at least 90 days after the procedure, and still menstruating or <1 year has passed since the last menses, if menopausal.*

*[5]Includes full abstinence from sexual intercourse with a male partner, monogamous relationship with vasectomized partner who has been vasectomized for 180 days or more or shown to be azoospermic prior to the participant receiving the study vaccination, barrier methods such as condoms or diaphragms/cervical cap, intrauterine devices, NuvaRing®, tubal ligation, and licensed hormonal methods such as implants, injectables or oral contraceptives ("the pill").*

15. Females 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.

16. For a female with potential to become pregnant, she understands that in the event of pregnancy during the study she will be asked to allow us to follow her during pregnancy through outcome.

17. Must agree to have blood collected, stored, and potentially used for auto-antibody studies (if a suspected PIMMC occurs in this participant).

### 5.1.2 Participant Exclusion Criteria

Participants eligible to participate in this trial must **not** meet any of the following exclusion criteria:

1. Have an acute illness[6], as determined by the site PI or appropriate sub-investigator, within 72 hours prior to study vaccination.

   [6]*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 and systemic reactogenicity events as required by the protocol.*

2. Have any medical disease or condition that, in the opinion of the site PI or appropriate sub-investigator, is a contraindication to study participation[7]

   [7]*Including acute, subacute, intermittent, or chronic medical disease or condition that would place the participant at an unacceptable risk of injury, render the participant unable to meet the requirements of the protocol, or may interfere with the evaluation of responses or the participant's successful completion of this trial.*

3. Have immunosuppression as a result of an underlying illness or treatment, a recent history or current use of immunosuppressive or immunomodulating disease therapy[8].

   [8]*These include oral or parenteral (including intra-articular) corticosteroids of any dose within 30 days prior to study vaccination, or high-dose inhaled corticosteroids within 30 days prior to study vaccination, with high-dose defined as per age as using inhaled high-dose per reference chart in the National Heart, Lung and Blood Institute Guidelines for the Diagnosis and Management of Asthma (EPR-3) or other lists published in UPTODATE. Intranasal corticosteroids are not exclusionary. Low and moderate potency topical corticosteroids are permitted.*

4. Use of anticancer chemotherapy or radiation therapy (cytotoxic) within 3 years prior to study vaccination.

5. Have known active or recently active (12 months) neoplastic disease or a history of any hematologic malignancy. Non-melanoma treated skin cancers are permitted.

6. Have known human immunodeficiency virus (HIV), chronic hepatitis B, or hepatitis C infection.

7. Have known hypersensitivity or allergy to any components of the study vaccines (AVA, CPG adjuvants, aluminum, benzethonium chloride [phemerol], formaldehyde).

8. Have a history of receipt or plan to receive, while enrolled in this study, a licensed or unlicensed anthrax vaccine (except for the vaccines under study herein).

9. Have a history of PIMMCs[9].

[9]*Refer to Appendix B*: *Adverse Events of Special Interest*

10. Have a history of alcohol or drug abuse within 5 years prior to study enrollment or test positive on the screening urine test for drugs of abuse[10].

[10]*Note that a prospective participant with a positive urine for drugs of abuse test for a medication reported by the participant and used to treat a condition that does not make them otherwise ineligible, may be permitted to enroll, at the discretion of the investigator or his designee.*

11. Have any diagnosis, current or past, of schizophrenia, bipolar disease, or other psychiatric diagnosis that may interfere[10] with participant compliance or safety evaluations.

[11]*As determined by the site PI or appropriate sub-investigator.*

12. Have been hospitalized for psychiatric illness, history of suicide attempt, or confinement for danger to self or others within 5 years prior to study vaccination.

13. Received or plan to receive a licensed, live vaccine within 30 days before or after each study vaccination.

14. Received or plan to receive a licensed, inactivated vaccine within 14 days before or after each study vaccination.

15. Have a known history of documented anthrax disease or suspected exposure to anthrax.

16. Received immunoglobulin or other blood products, except Rho(D) immunoglobulin, within 90 days prior to study vaccination.

17. Received an experimental agent[12]within 30 days prior to the study vaccination or expect to receive another experimental agent[13] during the trial-reporting period[14].

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

[13]*Other than from participation in this trial.*

[14]*Approximately 12 months after the second study vaccination.*

18. Are participating or plan to participate in another clinical trial with an interventional agent[15] that will be received during the trial-reporting period[16].

[15]*Including licensed or unlicensed vaccine, drug, biologic, device, blood product, or medication.*

[16]*Approximately 12 months after the second study vaccination.*

19. Female participants who are breastfeeding or plan to breastfeed from the time of the first study vaccination through 30 days after the second study vaccination.

20. Planning to donate blood within 4 months following second vaccination.

21. Planned elective surgery during study participation.

22. Member or immediate family member of the site research staff found on the delegation log.

23. Previously served in the military any time after 1990 and/or plan to enlist in the military at any time during the study.

### 5.1.3 Screen Failures

A screen failure is defined as a participant from whom informed consent is obtained and documented in writing, but who is not subsequently randomized to study treatment. Reasons for screen failure are to be recorded on the electronic case report forms (eCRFs).

All screening procedures must occur 2 to 28 days before the Day 1 study visit. On Day 1, participants are allocated to a treatment group and receive their first vaccination. The results of clinical laboratory tests at screening must be within the acceptable ranges for this study. Each clinical laboratory test for screening will not be performed more than twice (one initial and one repeat test for a total of two times). If the laboratory results are abnormal due to a processing or handling error, the test may be repeated without counting towards the not-to-exceed total of twice performed.

Participants who are screen failures are permitted to be rescreened one time (only) according to the PI's discretion. Otherwise eligible prospective participants may undergo additional rescreening visits in the event that they originally failed screening due to an eligibility criterion, such as a laboratory test, that is subsequently amended and does, upon amendment, then allow for inclusion. Such rescreening must occur in the time period before vaccination as specified in the protocol, and a single repeat of any laboratory test, as allowable during the original screening, is again allowable in this situation. In the event that the participant is rescreened for trial participation, a new informed consent form (ICF) must be signed. (This does not apply to those who are simply having an abnormal laboratory test repeated to determine eligibility.) For example, if there are any delays between screening and enrollment that cause eligible participants to fall outside the screening window, these participants may be re-consented and rescreened. Participants who complete the rescreening and are randomized in the study will not be considered screen failures.

## 5.2 Treatment Assignment Procedures

### 5.2.1 Enrollment and Randomization Procedures

Per ICH E6 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 (SDCC) Advantage eClinical[SM] (Electronic Data Capture System).

Once consented and upon entry of demographic data and confirmation of eligibility for this trial, the participant will be enrolled in the electronic data capture system. The participant will be enrolled and randomly assigned with equal allocation (1:1) to 1 of 2 treatment groups, stratified by age category (18-30 years old and 31-45 years old) and by gender.

Enrollment of participants will be done online using the enrollment module of Advantage eClinical[SM]. The randomization code will be prepared by statisticians at the SDCC and included in the enrollment module for this trial. Advantage eClinical[SM] will assign each participant to a treatment arm after the demographic and eligibility data have been entered into the system. A designated individual at the 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 Advantage eClinical[SM] User's Guide. Manual back-up procedures and instructions are provided for use in the event that the participating VTEU site temporarily loses access to the internet or the online enrollment system is unavailable.

### 5.2.2 Masking Procedures

This is a double-blinded (masked) clinical trial.

Participants, site investigators, and study personnel performing any study-related assessments following study vaccine administration are blinded to vaccine received. Laboratory personnel performing immunological assays will receive serum blinded to participant ID number, specimen visit number, and allocation group.

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.

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 participant contact for data collection following study vaccine administration.

The Safety Monitoring Committee (SMC) may receive data in aggregate and presented by treatment group. 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 participant if required for safety assessment. The SMC will review grouped and unblinded data in the closed session only.

### 5.2.3 Reasons for Withdrawals and Discontinuation of Study Product Administration

Participants may voluntarily withdraw their consent for trial participation at any time and for any reason, without penalty or loss of benefits to which they are otherwise entitled.

The site PI or appropriate sub-investigator may also withdraw a participant from receiving the study vaccine for any reason.

A participant 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 PI or appropriate sub-investigator, would compromise the safety of the participant, or would interfere with the participant's successful completion of this trial, or would interfere with the evaluation of responses.
- Participant withdrawal of consent or refusal to be re-vaccinated.
- Participant loss to follow-up.
- Termination of this trial.
- As deemed necessary by the site PI or appropriate sub-investigator for noncompliance or other reasons.
- New information becomes available that makes further participation unsafe.

### 5.2.4 Handling of Withdrawals and Discontinuation of Study Product Administration

The primary reason for withdrawal from this trial will be recorded on the Study Status electronic Case Report Form (eCRF). Participants will be encouraged to complete the Early Termination Visit. The Early Termination Visit procedures are listed in Section 8.4.

Every attempt will be made to follow all AEs, including solicited injection site and systemic reactions, unsolicited non-serious AEs, SAEs, MAAEs, AESIs (PIMMCs) ongoing at the time of early withdrawal through resolution as per applicable collection times defined for the specific type of AE.

In the case of participants who fail to appear for a follow-up safety assessment, extensive effort (i.e., three documented contact attempts via phone calls 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 per site protocol.

The site PI or appropriate sub-investigator will inform the participant that already collected data will be retained and analyzed even if the participant withdraws or is withdrawn from this study.

### 5.2.5 Participant Replacement

Participants who withdraw, or are withdrawn or terminated from this trial, or are lost to follow-up after signing the informed consent form (ICF), randomization, and receipt of study vaccine may be replaced. If a participant withdraws after signing the ICF, but before randomization and/or receipt of study vaccine, they may also be replaced. We do not believe that it is likely that there will be any such withdrawals, but, should replacement be required, the total number of persons enrolled may exceed 40 and approval for the overall number enrolled will be obtained from the IRB.

### 5.2.6 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 SMC review and recommendation, and at the discretion of DMID.

If this trial is prematurely terminated by the sponsor, any regulatory authority, the site PI, or appropriate sub-investigator for any reason, the site PI or appropriate sub-investigator will promptly inform the participants and assure appropriate therapy or follow-up for them, as

necessary. The site PI or appropriate sub-investigator will provide a detailed written explanation of the termination to the IRB.

# 6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

## 6.1 Study Product Description

AV7909 Liquid Formulation

AV7909 (Emergent BioSystems) is an investigational vaccine that is a preformulated, sterile, milky-white suspension (when mixed) for IM injection. It consists of the AVA bulk drug substance and CPG 7909 adjuvant. CPG 7909 is a white to off-white lyophilized powder manufactured by Avecia Nitto Denko (Avecia) in Milford, MA. CPG 7909's natural deoxyribonucleic acid (DNA) phosphodiester backbone has been modified to a phosphorothioate backbone, providing the molecule with increased stability and resistance to degradation for improved in vivo activity. The final base sequence of the full-length CPG 7909 is 24 nucleotides in length.

AV7909 Lyophilized Formulation

Thermostable AV7909 is a lyophilized version of the liquid AV7909 formulation which, after reconstitution, is a sterile, milky-white suspension for intramuscular injection. The Thermostable AV7909 Bulk Intermediate Product is manufactured at Emergent's Baltimore, MD facility by addition of Polysorbate 80 and CPG7909 adjuvant to AVA Bulk Drug Substance produced by Emergent's Manufacturing facility at Lansing, MI.

The Thermostable AV7909 Final Drug Product is manufactured by Integrity Bio Inc. The vaccine is supplied in a 10 mL Schott glass vial with a West 4432/50 Gray stopper treated with FluoroTec® fluorocarbon film and a 20 mm flip-top aluminum seal, stored at 2-8°C. After reconstitution with 4.0 mL Water For Injection, a single vial contains approximately 4.8 mL for a total of 8 doses per vial (0.5 mL each), with ~1.3 mg/mL aluminum, 0.45 mg/mL CPG7909, 0.01% formaldehyde, 22.5 ppm benzethonium chloride (phemerol), 0.025% Polysorbate 80, 0.9% NaCl and 25% trehalose dihydrate at a target pH of 8.0.

Acquisition

Both vaccine formulations will be provided by Emergent BioSolutions.

Diluent for the lyophilized formulation will also be provided by Fisher BioServices.

Upon request by DMID, both vaccines will be transferred 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

Study vaccine will be provided through the DMID CMS to the participating VTEU site prior to the start of this trial upon request and with prior approval from DMID. Should the site PI require additional vaccine or diluent, further instructions are provided in the protocol-specific MOP.

### 6.1.1 Formulation, Storage, Packaging, and Labeling

AV7909 Liquid Formulation

The AV7909 liquid formulation vaccine will be supplied in 6 mL (nominal fill volume) clear borosilicate glass multi-dose vials with rubber stoppers and flip-top aluminum seals for storage. A single vial is filled with approximately 6.1 mL. For purposes of this trial, only a single dose (0.5 mL) will be used from each vial.

AV7909 liquid formulation must be stored at 2-8 °C (36-46 °F). Do not freeze. Do not shake. Avoid foaming.

AV7909 Lyophilized Formulation

AV7909 lyophilized formulation must be stored between 2-8 °C (36-46 °F). Do not freeze.

The AV7909 lyophilized product is mixed with the diluent. The diluent is USP grade sterile Water For Injection. After reconstitution with 4.0 mL Water For Injection, a single vial contains approximately 4.8 mL. This volume can allow for up to 8 doses per vial (0.5 mL for each dose). For purposes of this trial, only a single dose (0.5 mL) will be used from each vial. The reconstituted product must be used within 24 hours of the time of reconstitution. If not used immediately, the reconstituted vial can be stored at 2-8°C.

Once the vaccine is pulled into a syringe, if not used immediately, the filled syringe must be stored at 2°C to 8°C (36°F to 46°F). Do not freeze. The storage time cannot exceed 24 hours.

Each of the study products will be labeled according to manufacturer specifications and include the statement “Caution: New Drug Limited by Federal Law to Investigational Use.”

Further details are included in the manufacturer’s Investigator Brochures for both vaccines.

Diluent for the Lyophilized Formulation

AV7909 lyophilized product will be diluted with USP grade sterile Water For Injection as described above. This product will be provided via Fisher BioServices and stored at 2°C to 30°C (36°F to 86°F).

### 6.1.2 Study Product Storage and Stability Procedures

The temperature of the storage unit must be manually recorded daily (excluding non-business days and holidays, as applicable) and continuously monitored and recorded during the course of this trial per the participating VTEU site 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 participating VTEU site's research pharmacist must alert the site PI and study coordinator if the temperature fluctuates outside of the required range. In the event the temperature fluctuates outside of the required range, including accidental deep-freezing or disruption of the cold chain, the affected study product(s) must not be administered. The site PI 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 and DMID contact information are provided in the protocol-specific MOP.

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

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

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.

Study vaccine administration to the participant 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 participant contact for data collection following study vaccine administration. One dose of study vaccine will be administered to the participant via a single IM injection into the deltoid muscle of the participant's preferred arm on the day of study vaccine administration. See the protocol-specific MOP for information on how to administer IM injections. The site of injection (right or left arm) and time of study vaccine administration will be recorded on the appropriate eCRF. The second study vaccination will be given in the opposite arm.

Preparation of the product will be performed as specified in detail in the protocol-specific MOP.

## 6.3 Accountability Procedures for the Study Intervention/Investigational Product

After receipt of the study vaccine(s), the site PI is responsible for study product distribution and disposition and has ultimate responsibility for study product accountability. The site PI may delegate responsibility for study product accountability to the participating VTEU site research pharmacist who will be responsible for maintaining complete records and documentation of study product receipt, accountability, dispensation, storage conditions, and final disposition of the study product(s). Study product accountability records and dispensing logs will also capture the date of study vaccine preparation/administration, time of study vaccine preparation, expiration 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 will be recorded on the appropriate eCRF. All study product(s), 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 study product accountability records and dispensing logs per the site monitoring plan. After a single dose is withdrawn from an AV7909 formulation vial (liquid or reconstituted lyophilized) for administration to a participant, the vial and any unused contents will thereafter be maintained at room temperature pending final drug accountability procedures.

## 6.4 Assessment of Participant Compliance with Study Intervention/Investigational Product

Study vaccine will be administered to the participant by an unblinded study vaccine administrator via IM injection at all dosing times per the participant's randomized treatment assignment and as described in Section 6.2. Study vaccine administration and any noncompliance with the dose schedule will be recorded on the appropriate eCRF.

## 6.5 Concomitant Medications/Treatments

Administration of any medications, therapies, or vaccines will be recorded on the appropriate eCRF. Concomitant medications will include all current medications and medications taken in the 60 days prior to signing the ICF through approximately Day 64 or early termination, whichever occurs first. Concomitant medications (new medications or changes to previously reported medications) that are reported after Day 64 will only be recorded when they are taken in relation to an MAAE, AESI (PIMMC), or SAE. Prescription and over-the-counter drugs will be included as well as herbals, vitamins, and supplements. Use of a new medication should prompt evaluation for the occurrence of any AE, MAAE, AESI (PIMMC), or SAE.

Medications that might interfere with the evaluation of the investigational product(s) should not be used during the trial-reporting period (approximately 12 months after the second study vaccination, approximately Day 380) unless clinically indicated as part of the participant's health care. Medications in this category include the prohibited medications per the Participant Exclusion Criteria (see Section 5.1.2). In addition, the site PI or appropriate sub-investigator may identify other medications, herbals, vitamins, or supplements that should not be used due to a risk to participant safety or assessment of reactogenicity and immunogenicity.

# 7 STUDY PROCEDURES/EVALUATIONS

## 7.1 Clinical Evaluations

A complete medical history will be obtained by interview of participants at the screening visit. Participants will be queried regarding a history of significant medical disorders of the head, eyes, ears, nose, throat, 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, immune deficiency, psychiatric illness, substance abuse, autoimmune disease, and any other significant medical history will be solicited. At all subsequent visits, an interim medical history will be obtained by interview of participants and any changes since the previous clinic visit or phone call will be noted, when within the respective reporting period. The interim medical history will include an assessment for new medical conditions, MAAEs, and symptoms suggestive of AESIs (PIMMCs).

Concomitant medications will be collected as described in Section 6.5.

At the screening visit, a physical examination will be performed on all participants, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator, to include the following organs and organ systems: abdomen, cardiovascular/heart, extremities, general appearance, HEENT, hepatobiliary/spleen, lymph nodes, musculoskeletal, neck, neurological, pulmonary/chest, and skin. Specific attention will be placed in assessing for physical signs of PIMMCs. At the vaccination visit and at follow-up visits after each study vaccination, a targeted physical examination may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator, if indicated, based on the participant's interim medical history. Targeted physical examinations should also include an assessment for signs suggestive of PIMMCs.

Height and weight will be collected at the screening visit for calculation of the BMI. Vital signs (oral temperature, pulse, and blood pressure) will be collected at the screening visit and prior to each study vaccination. Vital signs assessed on Day 1 prior to the first study vaccination will be considered as baseline. Participants must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking an oral temperature. A 12-lead electrocardiogram will be performed at screening and reviewed by an investigator and designated cardiologist.

Reactogenicity assessments will include an assessment of solicited AEs occurring for the week after each study vaccination (Days 1 through 8 and target of Days 15 through 22, inclusive), which includes an assessment of injection site reactions including pruritus, ecchymosis,

erythema, edema/induration, pain, and tenderness as well as systemic reactions including fever, feverishness, fatigue, malaise, myalgia (exclusive of the injection site muscle pain), arthralgia, headache, and nausea. If a reactogenicity event persists on Day 8 or 22 (or the last day of the second reactogenicity period, should the second vaccination occur after Day 15), it will be followed until resolution. Pre-administration reactogenicity assessments will be performed immediately prior to each study vaccination to establish baseline.

Participants 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 will be recorded on the appropriate eCRF prior to discharge from the clinic. The study vaccination site will also be examined on approximately Day 8 after the first vaccination and Day 22 after the second (or the last day of the second reactogenicity period, should the second vaccination occur after Day 15).

All participants will complete a memory aid using an eMemory Aid from the time of first study vaccination through Day 8 and the second vaccination through Day 22 (or the last day of the second reactogenicity period, should the second vaccination occur after Day 15). Memory aids will be completed online and reviewed by the study staff.

## 7.2 Laboratory Evaluations

Clinical laboratory evaluations and special assays are described below. Refer also to Sections 4 and 8 as well as Appendix A: Schedule of Study Procedures and Evaluations and Appendix B: Adverse Events of Special Interest.

### 7.2.1 Clinical Laboratory Evaluations

A serum pregnancy test (β hCG) will be sent with the screening laboratory specimens and a point-of-care urine pregnancy test will be performed on Days 1 and 15 (or the day of second vaccination should the second vaccination occur after Day 15), with results determined to be negative before randomization and vaccination for all females of childbearing potential. Results must be negative and known prior to randomization on Day 1 and administration of vaccine on Day 15 (or the day of second vaccination should the second vaccination occur after Day 15) to be eligible for participation in this trial and receipt of study vaccinations.

Clinical safety laboratory parameters will be collected at the screening study visit. The results from the clinical safety laboratory parameters collected at the screening visit will be reviewed prior to the first study vaccination and will be within acceptable ranges to be eligible for receipt of the first study vaccination. The safety laboratories will be repeated on approximately Day 29.

At screening, tests for HIV infection (fourth generation antigen-antibody test), acute and chronic hepatitis B infection (HBsAg), and hepatitis C infection (antibody) will be collected and must be negative for a participant to be deemed eligible. In addition, $HgbA_1C$ will be performed at screening and the value must fall within the protocol-specified eligibility range in order for prospective participants to join the study. A urine study for drugs of abuse must also be negative for prospective participants to be deemed eligible. Participants will be encouraged to review results, when abnormal, with their primary care physicians. These tests will not be routinely retested during the trial.

| **Test** | **Eligibility Criterion** |
|---|---|
| BUN | <23 mg/dL |
| Serum creatinine (female) | <1.3 mg/dL |
| Serum creatinine (male) | < 1.4 mg/dL |
| Alkaline phosphatase (female) | <147 U/L |
| Alkaline phosphatase (male) | <192 U/L |
| ALT (aka SGPT) | <68 U/L |
| Total bilirubin | < 1.3 mg/dL |
| Hemoglobin (female) | >10.9 g/dL |
| Hemoglobin (male) | > 12.4 g/dL |
| White blood cell count | 3000-12,000 cells/$mm^3$ |
| Absolute eosinophil count | <1201 cells/$mm^3$ |
| Absolute neutrophil count | >1200 cells/$mm^3$ |
| Platelets | >126,000 cells/$mm^3$ |
| Hemoglobin $A_1C$ | <6.5% |
| Urine for Drugs of Abuse* | All negative |
| HBsAg | Non-reactive |
| HCV antibodies | Negative |
| HIV $4^{th}$ generation test | Negative |

**amphetamines, barbiturates, benzodiazepines, cocaine, methadone, opiates, oxycodone/oxymorphone, phencyclidine (PCP), and propoxyphene. Note that a prospective participant with a positive urine for drugs of abuse test for a medication reported by the participant and used to treat a condition that does not make them otherwise ineligible, may be permitted to enroll, at the discretion of the investigator or his designee.*

The volume of venous blood to be collected for laboratory evaluations is presented in **Table 3.**

### 7.2.2 Special Assays or Procedures

Serological/Immunogenicity Assays

Serum will be collected for toxin neutralization assay (TNA) and reported as $ED_{50}$ and $NF_{50}$ and collected for anti-PA IgG concentration by ELISA and reported as µg/mL. Blood samples (sera) for determination of TNA titers and anti-PA IgG will be collected on the days outlined in the Schedule of Events in Appendix A. Specific procedures related to collection, processing, storage, and shipment of the samples are provided in the Manual of Procedures.

The TNA assay being used in this trial has been validated by Battelle Memorial Institute (Columbus, Ohio) under National Institute of Allergy and Infectious Diseases sponsorship {Clement, 2008}. The assay measures the functional ability of antisera containing anti-PA antibodies to specifically protect cells against *B. anthracis* lethal toxin cytotoxicity {Stinson et al, 2005; Li et al., 2008}. The TNA assay results will be reported as the reciprocal of a serum sample dilution that results in 50% neutralization of lethal toxin cytotoxicity (50% effective dilution; $ED_{50}$). To standardize assay results, the results are divided by the $ED_{50}$ of a serum reference standard, and the resulting ratio is reported as a 50% neutralization factor, $NF_{50}$. Reference standard AVR801 will be used.

The method of quantitative anti-PA IgG ELISA has been described previously (Semenova VA, Schiffer J, Steward-Clark E, Soroka S, Schmidt DS, Brawner MM, Lyde F, Thompson R, Brown N, Foster L, Fox S, Patel N, Freeman AE, Quinn CP J Immunol Methods. 2012 Feb 28; 376(1-2):97-107).

**Table 3: Venipuncture Volumes**

| Visit Number | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Cumulative Blood Volume Total (mL) |
|---|---|---|---|---|---|---|---|---|---|---|

| **Day#** | **Screening D-28 to -2** | **1** | **8** | **15** | **22** | **29** | **64** | **195** | **380** | |
|---|---|---|---|---|---|---|---|---|---|---|
| Vaccination | | X | | X | | | | | | |
| Serum pregnancy for women | 6 | | | | | | | | | **6** |
| Hematology | 6 | | | | | 6 | | | | **12** |
| Chemistry | 6 | | | | | 6 | | | | **12** |
| Hgb $A_1C$ | 6 | | | | | | | | | **6** |
| HIV, hepatitis B, hepatitis C | 12 | | | | | | | | | **12** |
| Stored Serum* | 10 | | | | | | | | | **10** |
| Serological Assays (TNA and anti-PA IgG) | | 20† | 20 | 20† | 20 | 20 | 20 | 20 | 20 | **160** |
| **Per Visit Blood Volume Total (mL)** | **46** | **20** | **20** | **20** | **20** | **32** | **20** | **20** | **20** | |
| **Running Blood Volume Total (mL)** | **46** | **72** | **92** | **112** | **132** | **164** | **184** | **204** | **224** | **218** |

# Note that Day 15, the visit at which the participant receives the second vaccination, has an acceptable window of Days 15 through 18, inclusive. Should the targeted Day 15 visit occur after Day 15, the subsequent dates are adjusted to keep the same intervals as in the Table above.

* This blood is drawn to store serum that could be used in the event of a post-vaccination PIMMC. For participants who agree to secondary use, the serum may be used for other secondary use purposes; for those who do not agree to secondary use, the serum will be discarded when required after study completion.

† Blood must be drawn prior to each study vaccination.

### 7.2.3 Specimen Preparation, Handling, and Shipping

#### 7.2.3.1 Instructions for Specimen Preparation, Handling, and Storage

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

#### 7.2.3.2 Specimen Shipment

Specimen shipment will occur at intervals during the course of this trial following all applicable International Air Transport Association (IATA) requirements and according to the specifics for storage temperature and documentation as detailed in the Central Clinical Laboratory Manual and protocol-specific MOP, as appropriate.

Specimens for clinical safety laboratory evaluations will be shipped from the participating VTEU site to the commercial laboratory except those done locally.

Specimens for TNA and ELISA will be shipped from the participating VTEU as outlined in the MOP.

Further instructions for specimen shipment are included in the protocol-specific MOP, as appropriate.

# 8 STUDY SCHEDULE

Complete study schedule details listed by type of visit are described below. Refer also to Sections 4 and 7 as well as Appendix A: Schedule of Study Procedures and Evaluations and Appendix B: Adverse Events of Special Interest.

## 8.1 Screening and Enrollment Visits

During recruitment, the prospective participant may provide to research team members personal information by phone or other means. This information may be used for screening and determining eligibility. The IRB will approve all methods of screening and recruiting along with any required documents or scripts, if used.

### 8.1.1 Visit 0, Screening (Day -28 to -2), Clinic Visit

- Participants will be provided with a description of this trial (purpose and study procedures) and asked to read and sign the ICF. The ICF will be signed prior to performing any study procedures.
- Demographic information will be obtained by interview of participants.
- Eligibility criteria will be reviewed with participants to ensure eligibility.
- Complete medical history will be obtained by interview of participants to ensure eligibility.
- All concomitant medications taken within 60 days prior to signing the ICF will be reviewed with participants and recorded to determine stability of chronic diseases and eligibility.
- Vital signs (oral temperature, pulse, and BP) will be obtained to ensure eligibility. Participants must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- Height and weight will be measured and recorded for the calculation of BMI.
- A physical examination will be performed on all participants to include the following organs and organ systems: abdomen, cardiovascular/heart, extremities, general appearance, HEENT, hepatobiliary/spleen, lymph nodes, musculoskeletal, neck, neurological, pulmonary/chest, and skin. Specific attention will be placed in assessing for

physical signs of PIMMCs. The examination will be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator.

- A serum pregnancy test will be performed for all females of childbearing potential. Results must be negative to ensure eligibility. Women of childbearing potential will be counseled to avoid pregnancy until 60 days after the second (final) vaccination, i.e., Day 75.

- Venous blood and urine will be collected for the tests outlined in Sections 7.2.1 and 7.2.2. The results must be within the acceptable range as outlined in the eligibility criteria to be eligible for randomization and vaccination.

- A 12-lead electrocardiogram will be performed and reviewed by the investigator and the designated cardiologist.

### 8.1.2 Visit 1, Day 1, Enrollment (Vaccination), Clinic Visit

- Participant's willingness to participate will be reconfirmed and documented in the study records prior to performing any further study procedures, including administration of study vaccination.

- Eligibility criteria, including results from screening lab tests, will be reviewed with participants prior to the first study vaccination to ensure continued eligibility.

- Interim medical history, including an assessment for new medical conditions, stability of chronic diseases and symptoms suggestive of PIMMCs, will be obtained by interview of participants prior to study vaccination. Any changes in medical history since the screening visit will be reviewed with participants prior to study vaccination to ensure continued eligibility.

- All concomitant medications will be reviewed with participants prior to study vaccination for accuracy and completeness. Any new concomitant medications taken since the screening visit will be reviewed with participants prior to study vaccination to ensure continued eligibility. Medications reported in the eCRF are limited to those taken within 60 days prior to the first study vaccination.

- Vital signs (oral temperature, pulse, and BP) will be obtained prior to study vaccination to ensure continued eligibility. Vital signs assessed on Day 1 prior to study vaccination will be considered as baseline. Participants must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

- A targeted physical examination, including an assessment for signs suggestive of PIMMCs, may be performed, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator, prior to study vaccination, if indicated based on review of complete medical history and any updates obtained by interview of participants since the screening visit. If not indicated, no physical examination is necessary.

- A urine pregnancy test will be performed locally by the site within 24 hours prior to study vaccination for all females of childbearing potential. Results must be negative and known prior to randomization and study vaccination. Women of childbearing potential will be counseled to avoid pregnancy until 60 days after the second (final) vaccination, i.e., Day 75.

- Venous blood will be collected prior to study vaccination for baseline serological assays (TNA and anti-PA ELISA).

- Participants will be randomized in Advantage eClinical$^{SM}$ and randomly assigned to a treatment arm prior to study vaccination.

- Pre-administration reactogenicity assessments will be performed immediately prior to study vaccination to establish baseline.

- Participants will then receive one dose of study vaccine via a single IM injection into the deltoid muscle of the participant's preferred arm. The site of injection (right or left arm) and time of study vaccine administration will be recorded on the appropriate eCRF. Participants will be observed in the clinic for at least 30 minutes after study vaccination. The study vaccination site will be examined, post-administration reactogenicity assessments will be performed, and any AE/SAEs will be recorded on the appropriate eCRF prior to discharge from the clinic.

- Participants will be trained to complete a web based "eMemory Aid" and are expected to enter information in the eMemory Aid each day. Participants using the eMemory Aid will also be provided a paper memory aid for their use in the event they are unable to access the web-based system. The participants will be asked to enter the information from the paper memory aid into the eMemory Aid once they are able to access the web-based system. Participants will be provided with instructions on completion of the electronic memory aid and other study-related materials. They will learn to record daily oral temperature, solicited injection site and systemic reactions, unsolicited AEs, and concomitant medications. Participants will be encouraged to take their oral temperature around the same time each day. They must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking their oral temperature. Participants will be

instructed on how to use their memory aid and how to measure and record AEs prior to discharge from the clinic. They will be instructed to notify the study center if they develop any severe reactions after study vaccination. If the site PI or appropriate sub-investigator deems the reaction severe enough, further instructions will be given to the participant on the proper course of action, including a return to the clinic for immediate evaluation if appropriate.

## 8.2 Follow-up Visits

Follow-up visits are scheduled in reference to the study vaccination date as indicated for each visit window.

### 8.2.1 Visit 2, Day 8, Clinic Visit (End of Solicited Reactogenicity Period after First Vaccination) (Window: Day 8 to Day 11, inclusive)

- Interim medical history, including an assessment for new medical conditions, stability of chronic diseases and symptoms suggestive of AESIs (PIMMCs), will be obtained by interview of participants and any changes since the previous clinic visit will be noted.
- All changes to concomitant medications will be recorded on the appropriate eCRF.
- All MAAEs and AE/SAEs will be recorded on the appropriate eCRF.
- eMemory Aid information will be reviewed with participants. If any reactogenicity events are on-going, they will be recorded as AEs.
- A targeted physical examination, including an assessment for signs suggestive of AESIs (PIMMCs), may be performed, if indicated based on review of interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator.
- The study vaccination site will be examined.
- Venous blood will be collected for serological assays (TNA and anti-PA ELISA).
- Women of childbearing potential will be counseled to avoid pregnancy until 60 days after the second (final) vaccination, i.e., Day 75.

### 8.2.2 Visit 3, Day 15, Clinic Visit (Second Vaccination) (Window: Day 15 to 18, inclusive)

- Interim medical history, including an assessment for new medical conditions, stability of chronic diseases and symptoms suggestive of AESIs (PIMMCs), will be obtained by interview of participants and any changes since the previous clinic visit will be noted.
- All changes to concomitant medications will be recorded on the appropriate eCRF.
- All MAAEs and AE/SAEs will be recorded on the appropriate eCRF.
- A targeted physical examination, including an assessment for signs suggestive of AESIs (PIMMCs), may be performed, if indicated based on review of interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator.
- Venous blood will be collected for serological assays (TNA and anti-PA ELISA).
- Criteria for receipt of the second vaccination will be reviewed with participants prior to the second study vaccination to ensure continued eligibility. These criteria are identical to the eligibility criteria, with the following exceptions: continued consent must be confirmed, age will not be re-confirmed, physical exam will not be required to determine good health, BMI will not be recalculated, screening labs will not be repeated, and screening electrocardiogram will not be repeated.
- Vital signs (oral temperature, pulse, and BP) will be obtained prior to study vaccination to ensure continued eligibility. Vital signs assessed on Day 1 prior to study vaccination will be considered as baseline. Participants must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- A urine pregnancy test will be performed locally by the site within 24 hours prior to study vaccination for all females of childbearing potential. Results must be negative and known prior to study vaccination. Women of childbearing potential will be counseled to avoid pregnancy until 60 days after the second (final) vaccination, i.e., Day 75.
- Pre-administration reactogenicity assessments will be performed immediately prior to study vaccination to establish baseline.
- Participants will then receive their second dose of study vaccine via a single IM injection into the deltoid muscle of the opposite arm of the first vaccination. The site of injection (right or left arm) and time of study vaccine administration will be recorded on the

appropriate eCRF. Participants will be observed in the clinic for at least 30 minutes after study vaccination. The study vaccination site will be examined, post-administration reactogenicity assessments will be performed, and any AE/SAEs will be recorded on the appropriate eCRF prior to discharge from the clinic.

- Participants will be re-trained to complete a web based eMemory Aid and are expected to enter information in the eMemory Aid each day. Participants using the eMemory Aid will also be provided a paper memory aid for their use in the event they are unable to access the web-based system. The participants will be asked to enter the information from the paper memory aid into the eMemory Aid once they are able to access the web-based system. Participants will be reminded to record daily oral temperature, solicited injection site and systemic reactions, unsolicited AEs, and concomitant medications. Participants will be encouraged to take their oral temperature around the same time each day. They must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking their oral temperature. Participants will be instructed on how to measure and record AEs prior to discharge from the clinic. They will be instructed to notify the study center if they develop any severe reactions after study vaccination. If the site PI or appropriate sub-investigator deems the reaction severe enough, further instructions will be given to the participant on the proper course of action, including a return to the clinic for immediate evaluation if appropriate.

- Note that all subsequent visits are based on the day that Visit 3 occurs. If it occurs on Day 15, all subsequent visits and windows are as written below. If it occurs after Day 15, subsequent visit days will be adjusted to maintain the intervals. For example, if Visit 3 occurs on Day 16, then the remaining target Days are 23, 30, 65, 196, and 381. The number of days in the accompanying acceptable windows for each visit will remain the same. The dates as written below reflect the schedule assuming that Visit 3 occurs on Day 15.

### 8.2.3 Visit 4, Day 22, Clinic Visit (End of Solicited Reactogenicity Period after Second Vaccination) (Window: Day 22 to 25, inclusive)

- Interim medical history, including an assessment for new medical conditions, stability of chronic diseases and symptoms suggestive of AESIs (PIMMCs), will be obtained by interview of participants and any changes since the previous clinic visit will be noted.

- All changes to concomitant medications will be recorded on the appropriate eCRF.

- All MAAEs and AE/SAEs will be recorded on the appropriate eCRF.

- eMemory Aid information will be reviewed with participants. If any reactogenicity events are on-going, they will be recorded as AEs.

- A targeted physical examination, including an assessment for signs suggestive of AESIs (PIMMCs), may be performed, if indicated based on review of interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator.

- The study vaccination site will be examined.

- Venous blood will be collected for serological assays (TNA and anti-PA ELISA).

- Women of childbearing potential will be counseled to avoid pregnancy until 60 days after the second (final) vaccination – Day 75.

### 8.2.4 Visit 5, Day 29, Clinic Visit (Window: Day 27 to 31, inclusive)

- Interim medical history, including an assessment for new medical conditions, stability of chronic diseases and symptoms suggestive of AESIs (PIMMCs), will be obtained by interview of participants and any changes since the previous clinic visit will be noted.

- All changes to concomitant medications will be recorded on the appropriate eCRF.

- All MAAEs and AE/SAEs will be recorded on the appropriate eCRF.

- A targeted physical examination, including an assessment for signs suggestive of AESIs (PIMMCs), may be performed, if indicated based on review of interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator.

- Venous blood will be collected for serological assays (TNA and anti-PA ELISA).

- Venous bloodwill be collected for standard clinical safety evaluations (tests outlined in Table 10).

- Women of childbearing potential will be counseled to avoid pregnancy until 60 days after the second (final) vaccination, i.e., Day 75.

### 8.2.5 Visit 6, Day 64, Clinic Visit (Window: Day 60-68, inclusive)

- Interim medical history, including an assessment for new medical conditions, stability of chronic diseases and symptoms suggestive of AESIs (PIMMCs), will be obtained by interview of participants and any changes since the previous clinic visit will be noted.
- All changes to concomitant medications will be recorded on the appropriate eCRF.
- All MAAEs and AE/SAEs will be recorded on the appropriate eCRF.
- A targeted physical examination, including an assessment for signs suggestive of AESIs (PIMMCs), may be performed, if indicated based on review of interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator.
- Venous blood will be collected for serological assays (TNA and anti-PA ELISA).
- Women of childbearing potential will be counseled to avoid pregnancy until 60 days after the second (final) vaccination, i.e., Day 75.

### 8.2.6 Visit 7, Day 195, Clinic Visit (Window: Day 188 to 202, inclusive)

- Interim medical history, including an assessment for new medical conditions, stability of chronic diseases and symptoms suggestive of AESIs (PIMMCs), will be obtained by interview of participants and any changes since the previous clinic visit will be noted
- All changes to concomitant medications will be recorded on the appropriate eCRF only if they occurred after Day 64 and in relation to an MAAE, SAE, or AESI (PIMMC).
- MAAEs, SAEs, and AESIs (PIMMCs) will be recorded on the appropriate eCRF.
- A targeted physical examination, including an assessment for signs suggestive of AESIs (PIMMCs), may be performed, if indicated based on review of interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator.
- Venous blood will be collected for serological assays (TNA and anti-PA ELISA).

## 8.3 Final Visit

### 8.3.1 Visit 8, Day 380, Clinic Visit (Window: Day 373 to 387, inclusive)

- Interim medical history, including an assessment for new medical conditions, stability of chronic diseases and symptoms suggestive of AESIs (PIMMCs) will be obtained by interview of participants and any changes since the previous clinic visit will be noted.
- All changes to concomitant medications will be recorded on the appropriate eCRF only if they occurred after Day 64 and in relation to an MAAE, SAE, or AESI (PIMMC).
- MAAEs, SAEs, and AESIs (PIMMCs) will be recorded on the appropriate eCRF.
- A targeted physical examination, including an assessment for signs suggestive of AESIs (PIMMCs) may be performed, if indicated based on review of interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator.
- Venous blood will be collected for serological assays (TNA and anti-PA ELISA).

## 8.4 Early Termination Visit (if needed)

The following activities will be performed at the Early Termination Visit on participants who withdraw, or are withdrawn or terminated from this trial:

- Interim medical history, including an assessment for new medical conditions, stability of chronic diseases and symptoms suggestive of AESIs (PIMMCs), will be obtained by interview of participants and any changes since the previous clinic visit will be noted.
- All changes to concomitant medications will be recorded on the appropriate eCRF if within the window.
- All MAAEs and AE/SAEs will be recorded on the appropriate eCRF, if within the windows.
- eMemory Aid information will be reviewed with participants, if not previously completed and reviewed.

- Vital signs (oral temperature, pulse, and BP) may be obtained if indicated. Participants must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

- A targeted physical examination, including an assessment for signs suggestive of AESIs (PIMMCs) may be performed, if indicated based on review of interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator.

- The study vaccination site will be examined, if the early termination falls from Days 1 to 8 after either study vaccination.

- Post-administration reactogenicity assessment will be performed, if the early termination falls from Days 1 to 8 after either study vaccination.

- Venous blood will be collected for clinical safety labs and auto-antibodies, if not previously drawn.

- Venous blood will be collected for serological assay (TNA and anti-PA ELISA).

## 8.5 Unscheduled Visit (if needed)

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

- Interim medical history, including an assessment for new medical conditions, stability of chronic diseases and symptoms suggestive of AESIs (PIMMCs) will be obtained by interview of participants and any changes since the previous clinic visit or phone call will be noted (if indicated).

- All changes to concomitant medications will be recorded on the appropriate eCRF, if within the window.

- All MAAEs and AE/SAEs will be recorded on the appropriate eCRF, if within the windows.

- eMemory Aid information will be reviewed with participants, if applicable.

- Vital signs (oral temperature, pulse, and BP) may be obtained if indicated. Participants must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

- A targeted physical examination, including an assessment for signs suggestive of AESIs (PIMMCs), may be performed, if indicated based on review of interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator.

- The study vaccination site will be examined, if the unscheduled visit falls from Days 1 to 8 after either study vaccination.

- Post-administration reactogenicity assessment will be performed, if the unscheduled visit falls from Days 1 to 8 after either study vaccination.

- Venous blood will be collected for clinical safety labs and serological testing (TNA and anti-PA ELISA), if not already performed.

# 9 ASSESSMENT OF SAFETY

## 9.1 Specification of Safety Parameters

Safety will be assessed by the frequency and severity of the following:

1. SAEs occurring from the time of the first study vaccination through approximately 12 months after the second study vaccination (approximately Day 380).

2. Solicited AEs – reactogenicity events occurring from the time of each study vaccination (Day 1) through Day 8 after each study vaccination (or until resolved):

   a) Injection site reactions including pruritus, ecchymosis, erythema, edema/induration, pain, and tenderness.

   b) Systemic reactions including fever, feverishness, fatigue, malaise, myalgia, arthralgia, headache, and nausea.

3. Clinical safety laboratory AEs occurring from the time of the first study vaccination through approximately Day 29. Parameters are found in Table 10.

4. Unsolicited AEs –non-serious unsolicited AEs occurring from the time of the first study vaccination through approximately Day 64.

5. AESIs (PIMMCs) and MAAEs occurring from the time of the first study vaccination through approximately Day 380.

**Table 4: Safety/Tolerability Assessment Timeline**

| **Safety/Tolerability Evaluation [#]** | **Day 1** | **Day 8** | **Day 15** | **Day 22** | **Day 29** | **Day 64** | **Day 195** | **Day 380** |
|---|---|---|---|---|---|---|---|---|
| SAEs | | | | | | | | |
| Solicited AEs* | | | | | | | | |
| Clinical Safety Lab AEs | | | | | | | | |
| Unsolicited non-serious AEs | | | | | | | | |
| AESIs (PIMMCs) | | | | | | | | |
| MAAEs | | | | | | | | |

*#Note that all visits after Visit 3 are based on the day that Visit 3 occurs. If it occurs on Day 15, all subsequent visits and windows are as written in the Table. If it occurs after Day 15, subsequent visit days will be adjusted to maintain the intervals. For example, if Visit 3 occurs on Day 16, then the remaining target Days are 23, 30, 65, 196, and 381. The dates as written in the Table reflect the schedule assuming that Visit 3 occurs on Day 15.*

**Solicited AEs may require recording beyond Days 8 and 22, if they persist.*

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

### 9.2.1 Adverse Events

**Adverse Event (AE):** ICH E6 (R2) GCP defines an AE as any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. The 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 a 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 injection site and systemic (subjective and quantitative) reactions, not meeting the protocol-defined criteria for SAEs, will be recorded on the appropriate eCRF. Information to be collected for unsolicited non-serious AEs includes event description, date of onset, licensed study physician's assessment of severity and relationship to study product or alternate etiology (if not related to study product) (assessed only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site PI or sub-investigator), date of resolution, seriousness, and outcome. AEs occurring during the trial-collection and reporting period will be documented appropriately regardless of relationship to study product. AEs will be followed through resolution.

Any medical condition that is present at the time that the participant 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 will be recorded as an AE.

AEs must be assessed for severity and relationship to study product (see definitions below). AEs 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 eCRF.

**Medically-Attended Adverse Events (MAAEs):** For each unsolicited AE experienced, the participant 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.

**Adverse Events of Special Interest (AESI):** An adverse event of special interest (serious or nonserious) is one of scientific and medical concern specific to the sponsor's product or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor can be appropriate. Such an event might warrant further investigation in order to characterize and understand it. Depending on the nature of the event, rapid communication by the trial sponsor to other parties (e.g., regulators) might also be warranted.

**Potentially Immune-Mediated Medical Conditions (PIMMCs):** PIMMCs constitute a group of AEs that includes diseases which are clearly autoimmune in etiology and other inflammatory and/or neurologic disorders which may or may not have autoimmune etiologies. PIMMCs are presented in Appendix B: Adverse Events of Special Interest. This is the only category of AESIs that will be collected for this trial.

**Protocol Specified AESIs:** The only protocol-specific AESIs are those PIMMCs listed in Appendix B.

**Severity of Event:** AEs will be assessed by a licensed study physician listed on the Form FDA 1572 as the site PI or sub-investigator using a protocol-defined grading system (see Sections 9.2.2 and 9.2.3). 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 participant'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 participant's daily activities and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

**Relationship to Study Product:** The licensed study physician's assessment of an AE's relationship to study product is part of the documentation process, but it 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. The relationship to study product must be assessed for AEs using the terms: related or not related. 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 AE. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the AE.

- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

### 9.2.2 Reactogenicity

Reactogenicity events are solicited 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 injection site and systemic (subjective and quantitative) reactions:

**Table 5: Injection Site Reactogenicity Grading**

| 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** if pain medication is used, it is Over the Counter (OTC) and used for less than 24 hours | Subject is aware of pain; there is interference with daily activity **or** OTC pain medication is used for more than 24 hours | Subject is aware of pain, **and** it prevents daily activity or pain requires prescription medication |
| Tenderness-experienced with touching the injection site | Subject is aware of pain, but it does not interfere with daily activity, **and** if pain medication is used, it is Over the Counter (OTC) and used for less than 24 hours | Subject is aware of pain; there is interference with daily activity **or** OTC pain medication is used for more than 24 hours | Subject is aware of pain, **and** it prevents daily activity or pain requires prescription medication |

| Injection Site Reaction | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) |
|---|---|---|---|
| Pruritus | Does not interfere with daily activity | Interferes with daily activity | Prevents daily activity or requires prescription medication |
| Ecchymosis (Bruising)* | Does not interfere with daily activity | Interferes with daily activity | Prevents daily activity |
| Erythema (Redness)* | Does not interfere with daily activity | Interferes with daily activity | Prevents daily activity |
| Edema (Swelling)/Induration (Hardness)* | 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, erythema and edema/induration as analyzed by measurement will be graded as follows:

### Table 6: Injection Site Reactogenicity Measurements

| 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 |
| Edema (Swelling)/Induration (Hardness)* | <20 mm | 20 mm – 50 mm | >50 mm |

* Will not be used as halting criteria.

### Table 7: Subjective Systemic Reactogenicity Grading

| Systemic (Subjective) | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) |
|---|---|---|---|
| Feverishness | No interference with daily activity | Some interference with daily activity | Significant interference, prevents daily activity |
| Fatigue | No interference with daily activity | Some interference with daily activity | Significant interference, prevents daily activity |
| Malaise | No interference with daily activity | Some interference with daily activity | Significant interference, prevents daily activity |

| Systemic (Subjective) | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) |
|---|---|---|---|
| Myalgia* | No interference with daily activity | Some interference with daily activity | Significant interference, prevents daily activity |
| Arthralgia* | No interference with daily activity | Some interference with daily activity | Significant interference, prevents daily activity |
| Headache | No interference with daily activity | Some interference with daily activity | Significant interference, prevents daily activity or headache requires prescription medication |
| Nausea | No interference with daily activity | Some interference with daily activity | Significant interference, prevents daily activity or nausea requires prescription medication |

* Not at injection site.

Oral temperature[#] will be graded as follows:

**Table 8: Quantitative Systemic (Oral Temperature) Reactogenicity Grading**

| Systemic (Quantitative) | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) |
|---|---|---|---|
| Fever* – oral[†] | 38.0°C – 38.4°C<br>100.4°F – 101.1°F | 38.5°C – 38.9°C<br>101.2°F – 102.0°F | >38.9°C<br>>102.0°F |

[#] Oral temperature assessed on Day 1 prior to the first 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.

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

### 9.2.3 Additional Adverse Event Severity Grading

Pulse and BP[#] will be graded as follows:

**Table 9: Pulse and BP Adverse Event Grading**

| Physiologic Parameter | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) |
|---|---|---|---|
| Bradycardia – beats per minute | 45 – 50 | 40 – 44 | <40 |

| | | | |
|---|---|---|---|
| Tachycardia – beats per minute | 101 – 130 | 131 – 155 | >155 |
| Hypotension (systolic) mmHg | 80 – 84 | 75 – 79 | <75 |
| Hypotension (diastolic) mmHg | 50 – 54 | 45 – 49 | <45 |
| Hypertension (systolic) mmHg | 141 – 155 | 156 – 160 | >160 |
| Hypertension (diastolic) mmHg | 91 – 100 | 101 – 110 | >110 |

[#] Pulse and BP assessed on Day 1 prior to the first study vaccination will be considered as baseline.

Clinical safety laboratory values will be graded as follows:

**Table 10: Clinical Safety Laboratory Adverse Event Grading**

| Panel and Analyte[a] | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) |
|---|---|---|---|
| **Serum Chemistry** | | | |
| Blood Urea Nitrogen (mg/dL) | 23–26 | 27–31 | >31 |
| Creatinine (mg/dL) female | 1.3–1.7 | 1.8–2.0 | >2.0 |
| Creatinine (mg/dL) male | 1.4–1.7 | 1.8–2.0 | >2.0 |
| Alkaline phosphatase (U/L) female | 147-196 | 197-294 | >294 |
| Alkaline phosphatase (U/L) male | 192-256 | 257-384 | >384 |
| ALT(U/L) | 68-113 | 114-225 | >225 |
| Total Bilirubin when accompanied by any grade ALT (mg/dL) | 1.3-1.6 | 1.7-2.0 | >2.0 |
| Total Bilirubin when not accompanied by any grade ALT (mg/dL) | 1.3-2.0 | 2.1-2.6 | >2.6 |
| **Hematology** | | | |
| Hemoglobin, female (g/dL) | 10.0-10.9 | 9.0-9.9 | <9.0 |
| Hemoglobin decrease from baseline value, female (g/dL) | 1.0–1.5 | 1.6–2.0 | >2.0 |
| Hemoglobin, male (g/dL) | 11.5-12.4 | 10.5-11.4 | <10.5 |
| Hemoglobin decrease from baseline value, male (g/dL) | 1.0–1.5 | 1.6–2.0 | >2. 0 |
| WBC (leukocytosis) (cell/$mm^3$) | 12,001–15,000 | 15,001–20,000 | >20,000 |
| WBC (leukopenia) (cell/$mm^3$) | 2,500–2999 | 1,500–2,499 | <1,500 |

| Panel and Analyte[a] | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) |
|---|---|---|---|
| Eosinophils (absolute eosinophilia) (cell/mm$^3$) | 1201-1500 | 1,501–5,000 | >5,000 |
| Neutrophil (neutropenia) (cell/mm$^3$) | 1001-1200 | 751-1,000 | <751 |
| Platelets (thrombocytopenia)(cell/mm$^3$) | 100,000–126,000 | 50,000-99,999 | <50,000 |

ALT = alanine aminotransferase; WBC = white blood cell.
[a] Laboratory normal reference ranges have been taken into consideration for the toxicity grading scale. If the laboratory provides a value that has additional significant digits beyond what is provided in the table, digits of 0-4 will be rounded down and digits of 5-9 will be rounded up, for purposes of delineating a grade.

### 9.2.4 Serious Adverse Events

**Serious Adverse Event (SAE)**: An AE or suspected adverse reaction is considered "serious" if, in the view of either the site PI (or appropriate sub-investigator) or sponsor, it results in any of the following outcomes:

- Death,
- A life-threatening AE*,
- 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 participant 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.

* Life-threatening AE: An AE is considered "life-threatening" if, in the view of either the site PI (or appropriate sub-investigator) or sponsor, its occurrence places the patient or participant at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.

SAEs will be:

- Assessed for severity and relationship to study product or alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site PI or sub-investigator.
- Recorded on the appropriate SAE form and entered into the eCRF.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site PI or sub-investigator.
- Reviewed and evaluated by the SMC (periodic review unless related), DMID, and IRB, as required.

### 9.2.5 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

The site PI or appropriate sub-investigator is responsible for recording all AE/SAEs that are observed or reported during this trial, regardless of relationship to study product. AE/SAEs, abnormal laboratory test values, or abnormal clinical findings will be collected, assessed, documented, reported, and followed appropriately, using a local laboratory as necessary. In determining eligibility, refer to Section 5.1 and the protocol-specific MOP.

## 9.3 Reporting Procedures

Solicited injection site and systemic reactogenicity events will be documented and reported from the time of each study vaccination through 7 days after each study vaccination. These recording intervals are Days 1 through 8, inclusive, and Days 15 through 22, inclusive. If reactogenicity events are still present on Days 8 or 22, they will continue to be recorded until resolved.

Clinical safety laboratory AEs will be documented and reported following the post-vaccination clinical safety laboratory studies collected on approximately Day 29.

Unsolicited non-serious AEs will be documented and reported from the time of the first study vaccination through approximately Day 64.

MAAEs, SAEs, and AESIs (PIMMCs) will be documented and reported from the time of the first study vaccination through approximately 12 months after the second study vaccination (the entire time the participant is followed from first vaccination until study completion), i.e., through approximately Day 380.

### 9.3.1 Serious Adverse Events

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: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

In addition to the SAE form, selected SAE data fields must also be entered into Advantage eClinical[SM]. 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 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 participant safety and protocol conduct.

At any time after completion of this trial, if the site PI or appropriate sub-investigator becomes aware of an SAE that is suspected to be related to study product, the site PI 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 site PI or appropriate sub-investigator, DMID, the Investigational New Drug (IND) sponsor, will report any suspected adverse reaction that is both serious and unexpected. DMID will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the AE. DMID will notify the FDA and all investigators (i.e., the participating VTEU site PI to whom the sponsor is providing drug under its IND(s) or under any PI'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 the 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 the FDA, DMID will submit to the 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 adverse 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 Reporting of Pregnancy

Upon awareness, pregnancies occurring in participants will be recorded on the Pregnancy Report eCRF. All pregnancies reported during the course of this trial will be followed to pregnancy outcome, if allowable to the participant. With the participant's permission, we will continue to follow the participant for safety and immune response. If the participant becomes pregnant after the first vaccination, the second vaccination will not be given.

## 9.4 Type and Duration of Follow-up of Participants after Adverse Events

Non-serious unsolicited AEs will be collected, assessed, and followed through resolution from the time of first study vaccination through approximately Day 64.

MAAEs, SAEs, and AESIs (PIMMCs) will be collected, assessed and followed from the time of the first study vaccination through resolution even if this extends beyond the trial-reporting period (approximately 12 months after the second study vaccination, i.e., approximately Day 380).

Resolution of an AE/SAE 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 eCRF.

## 9.5 Halting Rules

### 9.5.1 Individual Halting Rules

- Any participant experiences ulceration, abscess, or necrosis at the injection site from vaccination until 7 days later (Days 1 through 8, inclusively, for the first vaccination and Days 15 through 22, inclusively, for the second vaccination. Should the second vaccination occur after Day 15, the reactogenicity period will occur from the day of vaccination until 7 days later)

- Any participant experiences laryngospasm, bronchospasm, or anaphylaxis within 24 hours after administration of the study products

- Any participant experiences generalized urticaria (defined as urticarial lesions occurring at more than two body parts) within 72 hours after administration of study product.

- Any participant experiences an SAE (except for accident or trauma) after administration of the study product.

- Any participant experiences a grade 3 AE including lab, local, or systemic solicited reactogenicity events (excluding measured grades of ecchymosis, erythema, and edema/induration alone)

### 9.5.2 Study Halting Rules

Additional enrollment and study interventions/administration of study products in this trial will be halted for SMC review/recommendation if any of the following are reported after the first study vaccination:

- Any participant experiences ulceration, abscess or necrosis at the injection site in the week after vaccination (Days 1 through 8, inclusively, for the first vaccination and Days 15 through 22, inclusively, for the second vaccination; should the second vaccination occur after Day 15, the reactogenicity period will occur from the day of vaccination until 7 days later) that is considered related to study product administration.

- Any participant experiences laryngospasm, bronchospasm or anaphylaxis within 24 hours after administration of study product that is considered related to study product.

- Two or more participants experience generalized urticaria (defined as urticarial lesions occurring at more than two body parts) within 72 hours after administration of study product that is considered related to study product.

- Any participant experiences an SAE after administration of study product that is considered related to study product.

- Any participant develops a PIMMC after administration of study product from receipt of study product until last study visit (approximately Day 380).

- Three or more participants experience a grade 3 injection site or systemic solicited adverse event (excluding measured grades of ecchymosis, erythema, and edema/induration alone).

- Three or more participants experience the same grade 3 AE (unsolicited and laboratory abnormality), in the same HLGT by MedDRA coding, considered related to study product.

Grading scales for solicited injection site and systemic (subjective and quantitative) reactions are included in Section 9.2.2. Grading scales for clinical safety laboratory AEs are included in Section 9.2.3.

If any of the halting rules are met following any participant receipt of 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, study interventions, and administration of study products during this trial, as applicable.

The DMID Medical Monitor is empowered to stop enrollment and study vaccinations if AEs that meet the halting criteria are reported.

## 9.6 Safety Oversight

### 9.6.1 Independent Safety Monitor (ISM)

**For this clinical trial, an ISM is <u>not</u> required**. The site PI or appropriate sub-investigator will send to the DMID Medical Monitor a summary of the events and include the site PI assessments.

### 9.6.2 Safety Monitoring Committee

Safety oversight will be conducted by an SMC that is an independent group of experts that monitors participant safety and advises DMID. The SMC members will be separate and independent of study personnel participating in this trial and will not have scientific, financial, or other COI related to this trial. The SMC will consist of members with appropriate expertise to contribute to the interpretation of the data from this trial.

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 clinical, safety, reactogenicity, and immunogenicity data. Reports may include enrollment and demographic information, medical history, concomitant medications, physical assessments, clinical laboratory values, dosing compliance, solicited and unsolicited AEs, MAAEs, SAEs, and AESIs (PIMMCs). The SMC will review SAEs on a regular basis and ad hoc during this trial. The DMID Medical Monitor will be responsible for reviewing SAEs in real time.

The SMC will conduct the following reviews:

- An Organizational Meeting that occurs prior to participant enrollment
- *Ad hoc* when a halting rule is met, or DMID/SMC chair may convene an *ad hoc* meeting 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 AEs that meet the halting criteria are reported.
- Final review meeting: 6 to 8 months after database lock to review the cumulative unblinded safety and immunogenicity data for this trial. The data will be provided in a standard summary format. The SMC may be asked to provide recommendations in response to questions posed by DMID.

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 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 assignment be unblinded for an individual participant if required for safety assessment. The SMC will review grouped and unblinded data in the closed session only. 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.

# 10 CLINICAL MONITORING

## 10.1 Site Monitoring Plan

Site monitoring is conducted to ensure that the human participants' protections, study and laboratory procedures, study interventions/administration of study products, and data collection processes are of high quality and meet sponsor and ICH E6 GCP guidelines and applicable federal regulations, and that this trial is conducted in accordance with the protocol, protocol-specific MOP and applicable sponsor SOPs. DMID, the sponsoring agency, or its designee will conduct site-monitoring visits as detailed in the clinical monitoring plan. DMID-designated clinical monitors will verify that this trial is conducted, and data are generated, documented (recorded), and reported in compliance with the protocol, ICH E6 GCP guidelines, and applicable regulatory requirements. Clinical monitoring reports will be submitted to DMID.

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, ICFs, 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 PIs to discuss any problems and actions to be taken and document visit findings and discussions.

# 11 STATISTICAL CONSIDERATIONS

## 11.1 Introduction

The goal of this clinical study is to assess in healthy adults, 18-45 years of age, the safety, reactogenicity, and immunogenicity of two doses of AV7909 administered as either the thermostable lyophilized product or as the liquid product.

## 11.2 Study Hypotheses

This Phase I study is not designed to test a formal null hypothesis. Rather, it is intended to obtain sufficient data to obtain meaningful estimates of the immune response induced by either vaccine formulation, and to uncover any safety issues that occur at a sufficiently high rate that they might be observed in a study of this size. The sample size facilitates formal testing of selected hypotheses as discussed in Section 11.4.3, along with the probability of observing safety outcomes and the precision of immunogenicity outcomes.

## 11.3 Study Outcome Measures

Please refer to Study Outcome Measures outlined in Table 1.

## 11.4 Sample Size Considerations

Please refer to Study Design outlined in Section 4.

### 11.4.1 Study Population

The study population for this clinical trial includes males and non-pregnant females, 18-45 years of age, who are in good health and meet all eligibility criteria. The participants will be recruited from the general population at one participating VTEU site.

### 11.4.2 Participant Enrollment and Follow-up

Based on the accrual rates observed in similar studies, it seems reasonable to expect that the participating VTEU will be able to enroll this trial in a timely fashion, about 12 weeks from first participant vaccinated until the final participant receives his/her first vaccination.

A total of 40 participants will be enrolled. Assuming up to 10% drop out by Day 64, it is expected that at least 18 subjects per group will be available for the primary immunogenicity analysis.

### 11.4.3 Sample Size

While this study is not designed to test any specific null hypothesis, the following illustrates the precision and power that are available for select estimates and comparisons of interest, based on the planned sample size of N=20 participants per study arm.

Table 11 indicates the probability of observing one or more safety events, such as solicited injection site or systemic reactogenicity events or an unsolicited AE of a particular type, for a single treatment arm (N=20), and for all enrolled subjects (N=40).

**Table 11: Probability (%) to Detect Safety Events Emmes**

| Event Frequency | N = 20 | N = 40 |
|---|---|---|
| ≥10% Very Common | 87 | 98 |
| ≥1% Common | 18 | 33 |
| ≥0.1% Uncommon | 1 | 3 |
| ≥0.01% Rare | <0.1 | <0.1 |

Binomial confidence intervals (CI) are widest (have the least precision) when the response rate is 50%. Table 12 is presented to indicate the worst-case scenario for precision of observed exact (Clopper-Pearson) binomial confidence intervals.

**Table 12: Precision of Binomial Confidence Intervals**

| N | 95% CI |
|---|---|
| 20 | 27-73 |
| 40 | 34-66 |

For each of the primary immunogenicity objectives, a power analysis is provided below for testing the following hypotheses with the planned sample size, where $p_c$ = probability of response in comparator arm; where $p_e$ = probability of response in experimental arm.

Test for difference in proportion responders:
$H_0$: $p_c - p_e = 0$ – No difference in response probabilities
$H_1$: $p_c - p_e \neq 0$ – difference in response probabilities

Table 13 illustrates the minimum detectable differences in the probability of responding (e.g., attaining seroconversion or $NF_{50} \geq 0.56$ [seroprotection]) between participants who receive AV7909 as the lyophilized formulation or liquid formulation using a two-sided likelihood ratio test with 80% power and alpha = 0.05. It is assumed that up to 10% of subjects (N=2) will be excluded from the per-protocol analysis in each group. Seroconversion rates of 10% to 60% are considered.

**Table 13: Minimum Detectable Difference in the Probability of Response with 80% Power**

| N per Group | Probability of Response in Comparator Group A | 80% Power | |
|---|---|---|---|
| | | Minimal Detectable Difference | Probability of Response in Experimental Group B |
| N = 18 | 0.10 | 0.39 | 0.49 |
| | 0.20 | 0.43 | 0.63 |
| | 0.30 | 0.43 | 0.73 |
| | 0.40 | 0.42 | 0.82 |
| | 0.50 | 0.39 | 0.89 |
| | 0.60 | 0.35 | 0.95 |

## 11.5 Planned Interim Analyses

No interim analyses are planned.

### 11.5.1 Interim Safety Review

An interim safety review may include enrollment and demographic information, medical history, concomitant medications, physical assessments, clinical laboratory values, dosing compliance, and solicited and unsolicited AE/SAEs. 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 treatment arm, including expected and observed rates of the expected AEs. The SMC will review grouped 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.

Additionally, this trial will be monitored to determine if any of the halting rules described in Section 9.5 are met.

## 11.6 Final Analysis Plan

Participant unblinding will occur and the final CSR will be completed after the last participant's last visit is completed, the final clinical database including all long-term safety follow-up data is cleaned, monitored and locked, and all primary and secondary immunogenicity data are available. There are no planned exploratory immunogenicity endpoints. If any are added via protocol amendment, then the additional exploratory immunogenicity endpoint data not available at the time of CSR preparation may be included in an addendum to the CSR.

A formal statistical analysis plan which defines the analyses to be included in the expedited report and the final CSR will be developed, finalized, and submitted to the FDA, prior to unblinding for any analysis.

### 11.6.1 Analysis Populations

The Safety Analysis population includes all participants who received the first dose of study vaccine.

The modified intent-to-treat (mITT) population includes all randomized participants who received the first dose of study vaccine and contributed at least one post-first study vaccination venous blood sample for immunogenicity testing for which valid results were reported. For

analyses using the mITT population, participants will be grouped based on randomized treatment arm.

The per protocol (PP) population includes all participants in the mITT with the following exclusions:

- Data from all available visits for participants found to be ineligible at baseline.
- Data from all visits for participants that did not contribute venous blood samples for immunogenicity testing.
- Data from all visits subsequent to major protocol deviations, such as:
    - Second vaccination not received
    - Second vaccination received out of window
    - Receipt of immunosuppressive therapy (e.g., corticosteroids) within 30 days before or after the first study vaccination.
    - Receipt of non-study licensed live vaccine within 30 days before or after each study vaccination.
    - Receipt of non-study licensed, inactivated vaccine within 14 days before or after each study vaccination.
- Data from any visit that occurs substantially out of window.

For analyses using the PP population, participants will be grouped based on study vaccinations received.

### 11.6.2 Safety Data

Summaries and analysis of safety data will be presented for the Safety Analysis Population. All summaries and analyses will be presented for all participants.

Solicited AEs will be summarized by severity after each study vaccination (through 7 days post study vaccination, Days 1 through 8 and Days 15 through 22) and as the maximum severity over all days. Additionally, solicited AEs will be analyzed by taking the most severe response over the follow-up period, dichotomizing into a binary variable (none versus mild, moderate, or severe) and using standard techniques, such as exact confidence intervals, to summarize the proportion of subjects reporting each symptom, any injection site symptom, and any systemic symptom. Summaries of solicited AEs will be presented for each study vaccination by treatment arm. The proportion of participants reporting symptoms may be compared between treatment arms using Chi-square or Fisher's exact test.

Unsolicited AEs will be coded by Medical Dictionary for Regulatory Activities (MedDRA®) for preferred term and system organ class (SOC). The numbers of SAEs, MAAEs, and AESIs

(PIMMCs) are likely to be small in this trial and will be reported by detailed listings showing the event description, MedDRA® preferred term and SOC, relevant dates (study vaccinations and AEs), severity, relatedness, and outcome for each event. Non-serious unsolicited AEs will be summarized as number and percentage of participants reporting at least one event in each MedDRA® preferred term and SOC, cross tabulated by severity and relationship to study product. Additionally, the proportion of participants and exact 95% confidence intervals of AEs in aggregate and by MedDRA® categories will be computed.

Clinical laboratory data will be summarized by severity for each visit and as the maximum over all post-study vaccination visits. Graphical presentations may include box plots.

### 11.6.3 Humoral Immunogenicity Data

Summaries and analysis of immunogenicity data will be presented for the mITT and PP populations; the PP population will be considered the primary analysis. Immune responses in terms of TNA $ED_{50}$ and $NF_{50}$, and anti-PA IgG by ELISA will be summarized by treatment arm at each time point. Analysis at all time points will include geometric mean titers (GMTs) and proportion of participants with seroconversion (defined as ≥4-fold increase over baseline levels, or a ≥4-fold increase over the LLOQ if the baseline value is <LLOQ). Additionally, the proportion of subjects in each study group with putative seroprotection (defined as $NF_{50}\geq 0.56$) will be assessed at each time point. All summaries will include the corresponding 95% confidence interval. No exploratory immunologic outcomes are anticipated.

### 11.6.4 Exploratory Immunogenicity Data

None.

### 11.6.5 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.

# 12 DATA COLLECTION FORMS AND ACCESS TO SOURCE DATA/DOCUMENTS

The participating VTEU site will maintain appropriate medical and research records for this clinical trial, in compliance with ICH E6 GCP Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of participants. The participating VTEU site will permit the study monitor or other authorized representatives of DMID as well as governmental regulatory agencies, such as the FDA, to examine (and when required by applicable law, to copy) clinical trial records for the purposes of quality assurance reviews, audits, monitoring and evaluation of the study safety and progress. These representatives will be permitted access to all source data, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, 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 participant files, and records kept at the pharmacy, at the laboratories and medico-technical departments involved in this clinical trial. Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the clinical trial.

Interview of participants is sufficient for obtaining medical history. Solicitation of medical records from the participant's primary care provider is not required.

The study uses direct data entry for the participating clinic site and a web-based electronic Memory Aid (eMemory Aid) for participants. All eCRFs serve as the source documents. Participants will be trained to use a database to complete a web based "eMemory Aid" and are expected to enter information in the eMemory Aid each day. Participants using the eMemory Aid will also be provided a paper memory aid for their use in the event they are unable to access the web-based system. The participants will be asked to enter the information from the paper memory aid into the eMemory Aid once they are able to access the web-based system.

Participants will record temperature, local and systemic symptoms, and if any new medications were used following vaccination or changes to previously reported medications daily for 8 days after any vaccination (Days 1 through 8 and Days 15 through 22).

Participants will be instructed to contact the clinic staff immediately if they experience significant symptoms at any time during the study, for prompt follow-up in real time. The study clinic will be alerted in real time of any potential solicited events of Grade 3 severity entered in the eMemory Aid. An email alert will be sent to the clinic site and the Emmes study team. Within one business day of site awareness, the site must attempt to follow up with the participant

on the severe solicited event and send an email to Emmes confirming attempted follow up with the participant. Instructions for completing the eMemory Aid are provided in the MOP and in a separate eMemory Aid instructions document that will be provided to the participants at each vaccination. The site staff must review the eMemory Aid information and interview the participant at the next scheduled visit. The participant-entered data will be included in the clinical database on the Solicited Events eCRF and will be available for review by the clinician during the clinical interview. All participant-entered data are to undergo clinical review.

Site staff who are delegated the responsibility by the study PI will be the data originators for clinical data entered directly into the eCRF in Advantage eClinical that will be used for the study endpoints. The central laboratory will be the data originator for laboratory data reported by an automated reporting system. A list of all authorized data originators, including site staff, will be included on the Study Personnel/Signature Responsibility List.

# 13 QUALITY CONTROL AND QUALITY ASSURANCE

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

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

# 14 ETHICS/PROTECTION OF HUMAN PARTICIPANTS

## 14.1 Ethical Standard

The site 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 site PI will also ensure conformity with ICH E6 GCP and applicable federal regulations, guidance and guidelines for GCP and Clinical Trials with humans.

## 14.2 Institutional Review Board (IRB)

The institution engaged in this research will hold a current Federal Wide Assurance (FWA) issued by the Office for Human Research Protections (OHRP) for federally funded research. The IRB must be registered with OHRP [OHRP-only or OHRP/FDA] as applicable to the research. The IRB FWA number will be provided to DMID.

The site PI will obtain IRB approval for this protocol to be conducted at his/her research site(s) and send supporting documentation to DMID before initiating recruitment of participants. The site PI will submit applicable information to the IRB on which it relies for the review, to conduct the review in accordance with 45 CFR 46, ICH E6 GCP guidelines, and as applicable, 21 CFR 56 (Institutional Review Boards), 21 CFR 50 (Protection of Human Subjects), and other federal, state, and local regulations and guidance. IRB approved recruitment process, screening script, and materials for participants may be utilized. DMID must receive the documentation that verifies IRB approval for this protocol, associated informed consent documents, and upon request, any recruitment material and handouts or surveys intended for the participants, prior to the recruitment and enrollment of participants.

Any amendments to the protocol or consent materials will be approved by the IRB before they are implemented. IRB review and approval will occur at least annually throughout the enrollment and follow-up of participants and may cease if annual review is no longer required by applicable regulations. The site PI will notify the IRB of protocol deviations and reportable SAEs in accordance with IRB requirements.

## 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. During recruitment, potential participants may be screened for eligibility by phone or email before obtaining written consent, per an IRB-approved process that ensures confidentiality. At the first clinic visit, before any study procedures are performed, informed consent will be obtained and documented. Participants will receive a concise and focused presentation of key information about the trial, verbally and with a written ICF. The explanation will be organized and presented in lay terminology and language that facilitates understanding why one might or might not want to participate. The ICF must not include any exculpatory statements.

The site PI or his designees will describe the protocol to potential participants face-to-face. The key information about the purpose of the trial, the procedures and experimental aspects of the trial, risks and discomforts, any expected benefits to the participants, and alternative treatment will be presented first to the participant.

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

Participants 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. Participants 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. Participants will be informed of the anticipated financial expenses, if any, for participating in the trial, as well as any anticipated prorated payments, if any, to the participant for participating in the trial. They will be informed of whom to contact (e.g., the site PI) for answers to any questions relating to the research project.

Information will also include the foreseeable circumstances and/or reasons under which the participant's participation in the trial may be terminated. The participants 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 he or she is otherwise entitled.

The extent of the confidentiality of the participant's records will be defined, and participants will be informed that applicable data protection legislation will be followed. They will be informed that the monitors, auditors, IRB, NIAID, and regulatory authorities will be granted direct access to their original medical records for verification of trial procedures and/or data without violating the confidentiality of the participant, to the extent permitted by the applicable laws and regulations, and that, by signing a written ICF, the participant is authorizing such access. Participants will be informed that records identifying them 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 participant's identity will remain confidential. Participants will be informed whether private information collected from this research and/or samples/specimens will be used for additional research, even if identifiers are removed. Participants will be allowed sufficient time to consider participation in the trial and have the opportunity to discuss the trial with their family, friends, or legally authorized representative, or think about it prior to agreeing to participate.

ICFs will be IRB-approved and participants will be asked to read and review the ICF. Participants must sign the ICF prior to starting any study procedures being done specifically for the trial. Once signed, a copy of the ICF will be given to the participants for their records. The participant(s) may withdraw consent at any time throughout the course of the trial. Their rights and welfare 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 or withdraw from the trial.

New information that significantly impacts the participant's risk of receiving the study interventions/study products will be communicated by the site PI or his/her designees to the participants who consent to participate in the trial in accordance with IRB requirements. The ICF will be updated and participants will be re-consented in accordance with IRB requirements, if necessary. Participants will be given a copy of all ICFs that they sign.

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

This trial will be inclusive of all interested persons, 18 to 45 years of age, who meet the Participant Inclusion Criteria (see Section 5.1.1) and do not meet the Participant Exclusion Criteria (see Section 5.1.2), 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 AV7909 poses any risks to an unborn child. Females of childbearing potential must utilize a highly effective method of contraception that is defined as one that results in a low failure rate (i.e., less than 1% per year) when used consistently and correctly (effective methods of birth control are outlined in the inclusion criteria, section 5.1.1). In addition to contraceptive use, all females of childbearing potential will be required to have a negative pregnancy test within 24 hours prior to each study vaccination. Female participants who become pregnant while participating in this trial will be asked to consider allowing the study team to follow them during pregnancy and record the outcome.

Children will not be included in this trial.

## 14.5 Participant Confidentiality

Participant confidentiality is strictly held in trust by the site PI, other study personnel, the sponsor, and their agents. This confidentiality includes documentation, investigation data, participant's clinical information, and all other information generated during participation in this trial. No information concerning this trial, or the data generated from this trial will be released to any unauthorized third party without written consent of the participant and approval by DMID.

Participant confidentiality will be maintained when trial results are published or discussed in conferences and is extended to cover testing of samples/specimens. The study monitor or other authorized representatives of DMID as well as governmental regulatory agencies, such as the FDA, may inspect all documents and records required to be maintained by the site PIs. This includes, but is not limited to, medical records (office, clinic or hospital) and pharmacy records for the participants in this trial. The participating VTEU 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 samples/specimens, evaluation forms, reports, and other records that leave the site will be identified only by a coded number and will not be identified by the participant's name.

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 participant, 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 participant, except as explained below.

The Certificate cannot be used to resist a demand for information from personnel of the US Government that is used for auditing or evaluation of federally funded projects, like this trial, or for information that must be released in order to meet the requirements of the FDA.

A Certificate of Confidentiality does not prevent the participant 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 participant's consent, information that would identify the participant 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 this trial is prematurely terminated by the sponsor, any regulatory authority, the site PI, or appropriate sub-investigator for any reason, the site PI or appropriate sub-investigator will promptly inform the participants and assure appropriate therapy or follow-up for them, as necessary. The site PI or appropriate sub-investigator will provide a detailed written explanation of the termination to the IRB. If any participant's private information will continue to be collected for this trial, the IRB must approve an ICF with the study procedures, any risks and discomforts as well as applicable elements, and the site PI or designee will re-consent the participants as approved by the IRB.

If this trial is discontinued, participants who have signed the ICF and are randomized and vaccinated will continue to be followed for safety for the duration of the prescribed safety follow-up period. No further study vaccinations will be administered.

## 14.7 Costs, Participant Compensation and Research Related Injuries

There is no cost to participants for the research tests, study procedures/evaluations or study vaccines while taking part in this trial.

Participants may be compensated for their participation in this trial. Compensation will be in accordance with local IRB requirements, and subject to local IRB approval.

If it is determined by the participating VTEU site and the site PI that an injury occurred to a participant 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 participant. Study personnel will try to reduce, control, and treat any complications from this trial. Immediate medical treatment may be provided by the participating VTEU site, such as giving emergency medications to stop immediate allergic reactions to the study vaccine. No financial compensation will be provided to the participant by NIAID, NIH or the participating VTEU site for any injury suffered due to participation in this trial.

**Public Readiness and Emergency Preparedness Act**

This protocol and the vaccine tested are covered under the Public Readiness and Emergency Preparedness (PREP) Act. The PREP Act provides compensation to participants in the event of serious physical injury or death caused by covered drugs and vaccines, and liability protection for persons conducting the clinical study and the manufacturer of the drug or vaccine.
The vaccine used in this clinical study is covered as a countermeasure under the PREP Act. This provides immunity for covered persons (including manufacturers, distributers, program planners, and other qualified persons who prescribe, administer, or dispense the vaccine) from tort liability, unless the injury was caused by willful misconduct.

The Act authorizes an emergency fund administered by the Health Resources and Services Administration via the Countermeasures Injury Compensation Program (CICP). The CICP provides 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 with the CICP within 1 year of administration or use of the countermeasure. For more information please visit the CICP website at http://www.hrsa.gov/cicp/. It is also advised participants consult an attorney in the event they suffer a serious injury and wish to file a claim for compensation with the CICP.

## 14.8 Secondary Use of Stored Specimens

Participants who agree to participate in this trial will have venous blood collected for clinical safety laboratory evaluations, stored samples for use in the evaluation of suspected or confirmed PIMMCs, and serological immunology assays. Any excess aliquots of serum available from blood samples, once protocol-defined assays are completed, will be stored for use in new or different immunological laboratory tests, to provide information for the development of new vaccines, or for the studies of anthrax or other infections. These aliquots, other than the blood drawn specifically for evaluation of PIMMCs, are derived from blood already collected as part of the study, not from additional blood volumes collected solely for the purpose of secondary use.

Secondary research use specimens, upon written request and approval from DMID, may be shared with investigators at the participating VTEU site, with other investigators or designated research laboratories for purposes of conducting additional immunological assessments other than PP analysis. The IRB will approve the use and breadth of secondary use specimens. DMID would authorize shipment from the DMID CMS. There are no benefits to participants in the collection, storage, and secondary research use of their samples/specimens. Secondary research use samples/specimens will not be sold or used directly for production of any commercial product. No genetic tests will be performed on stored/secondary use specimens. Samples will be stored indefinitely at an NIH-designated research storage facility.

Each sample/specimen will be encoded (labeled) only with a barcode and a unique tracking number to protect participant confidentiality. Reports from secondary research studies performed using participants' samples/specimens will NOT be kept in their health records and will not be reported to the participant. Assaying of secondary use specimens and providing reports of secondary use results will not be done during this protocol.

Participants will be asked to consent to the secondary research use of excess/residual specimens as an option, when they first consent to study participation. If they choose not to provide permission for excess blood and secondary use, they will still remain eligible for randomization and enrollment into the study.

Participants who at first choose to allow for secondary use of excess/residual aliquots may withdraw permission to use samples for secondary use at any time, even if they do not withdraw consent for participation. Samples stored for secondary use will not be assayed for secondary use tests during this study. Participants who choose to withdraw permission for secondary use will need to contact the study site and the samples will be removed from the study repository after this study is completed. Documentation will be completed that outlines the reason for withdrawal of permission for secondary use of samples.

There are no additional risks accrued by permitting secondary use of aliquots for the testing herein described. The scope of tests to be performed is similar in nature and detail to the immunologic tests already proposed in this protocol. The alternative is for participants to choose not to allow for secondary use of their stored specimens.

# 15 DATA HANDLING AND RECORD KEEPING

The site PI is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported.

Data will be directly entered into the case report forms by study personnel or received by direct data transfer. Data will be entered into an eCRF via a 21 CFR 11-compliant Internet Data Entry System provided and maintained by the Study Data Coordinating Center (SDCC). The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

For any forms requiring paper, data collection forms (DCFs) will be derived from the eCRF and provided by the SDCC to record and maintain data for each participant enrolled in this trial. All DCFs will be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making a change or correction, research staff members will cross out the original entry with a single line and initial and date the change. They will not erase, overwrite, or use correction fluid or tape on the original.

Electronic case report forms will serve as the source documents for data collected and will be created by the SDCC to record and maintain data for each participant enrolled in this study.

The sponsor and/or its designee will provide guidance to the site PIs and other study personnel on making corrections to the DCFs and eCRF. Details on data handling procedures, procedures for data monitoring, and instructions for use of the system and completion of the eCRFs are provided in the study MOP, eCRF Instructions, and Advantage eClinical User's Guide.

## 15.1 Data Management Responsibilities

All eCRFs and laboratory reports will be reviewed by the clinical team and data entry personnel, who will ensure that they are accurate and complete. AEs will be recorded on the appropriate eCRF, assessed for severity and relationship, and reviewed by the site PI or appropriate sub-investigator.

Data collection is the responsibility of the study personnel at the participating VTEU site under the supervision of the site PI. During this trial, the site PI will maintain complete and accurate documentation for this trial.

The SDCC for this trial 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, AESIs [PIMMCs], concomitant medications, medical history, physical assessments, and clinical laboratory values), reactogenicity, and immunogenicity data will be entered into a 21 CFR 11-compliant Internet Data Entry System provided and maintained by the SDCC. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete or inaccurate. Clinical and reactogenicity data will be entered directly onto eCRFs by the study personnel.

## 15.3 Types of Data

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

## 15.4 Timing/Reports

The final CSR will be completed after the last participant's last visit is completed, the final clinical database including all long-term safety follow-up data is cleaned, monitored and locked, and all primary and secondary immunogenicity data are available. There are no planned exploratory immunogenicity endpoints. If any are added via protocol amendment, then the additional exploratory immunogenicity endpoint data not available at the time of CSR preparation may be included in an addendum to the CSR.

A formal statistical analysis plan will be developed, finalized, and submitted to the FDA, prior to unblinding for any analysis, which defines the analyses to be included in the expedited report and the final CSR.

Additional statistical reports may be generated as deemed necessary and appropriate by DMID. Safety and immunogenicity summary reports may be generated for the SMC.

After the final CSR 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 participant treatment assignment. In this regard, the participating VTEU site requesting such information to share with participants must do so in accordance with IRB requirements.

## 15.5 Study Records Retention

Study records and reports, including, but not limited to, eCRFs, source documents, ICFs, and study drug disposition records shall be maintained for 2 years after a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for the drug, until 2 years after the investigation is discontinued and the FDA has been notified. These documents will be retained for a longer period, however, if required by local regulations. ICFs for secondary research use will be maintained as long as the sample/specimen exists.

No records will be destroyed without the written consent of the sponsor. It is the responsibility of the sponsor to inform the site PI when these documents no longer need to be retained. The participating VTEU sites must contact DMID for authorization prior to the destruction of any study records.

## 15.6 Protocol Deviations

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

These practices are consistent with ICH E6 GCP guidelines:

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 PI and other study personnel to use continuous vigilance to identify and report protocol deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. All protocol deviations must be promptly reported to DMID, via the SDCC's Advantage eClinical$^{SM}$.

All protocol deviations, as defined above, must be addressed on the appropriate eCRF. A completed copy of the Protocol Deviation eCRF must be maintained in the regulatory file as well as in the participants' chart. Protocol deviations must be sent to the IRB in accordance with IRB requirements. The site PI and other study personnel are responsible for knowing and adhering to 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 all 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 Office of Extramural Research (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 participant. 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 clinical trial, the responsible party is DMID which will register this 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

1. Centers for Disease C, Prevention. Cutaneous anthrax associated with drum making using goat hides from West Africa--Connecticut, 2007. MMWR Morb Mortal Wkly Rep 2008;57:628-31.
2. Inglesby TV, Henderson DA, Bartlett JG, et al. Anthrax as a biological weapon: medical and public health management. Working Group on Civilian Biodefense. JAMA 1999;281:1735-45.
3. Jernigan DB, Raghunathan PL, Bell BP, et al. Investigation of bioterrorism-related anthrax, United States, 2001: epidemiologic findings. Emerg Infect Dis 2002;8:1019-28.
4. Fellows PF, Linscott MK, Ivins BE, et al. Efficacy of a human anthrax vaccine in guinea pigs, rabbits, and rhesus macaques against challenge by Bacillus anthracis isolates of diverse geographical origin. Vaccine 2001;19:3241-7.
5. Brachman PS, Gold H, Plotkin SA, Fekety FR, Werrin M, Ingraham NR. Field Evaluation of a Human Anthrax Vaccine. Am J Public Health Nations Health 1962;52:632-45.
6. Wright JG, Quinn CP, Shadomy S, Messonnier N, Centers for Disease C, Prevention. Use of anthrax vaccine in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009. MMWR Recomm Rep 2010;59:1-30.
7. Hopkins RJ, Daczkowski NF, Kaptur PE, et al. Randomized, double-blind, placebo-controlled, safety and immunogenicity study of 4 formulations of Anthrax Vaccine Adsorbed plus CPG 7909 (AV7909) in healthy adult volunteers. Vaccine 2013;31:3051-8.
8. Minang JT, Inglefield JR, Harris AM, et al. Enhanced early innate and T cell-mediated responses in subjects immunized with Anthrax Vaccine Adsorbed Plus CPG 7909 (AV7909). Vaccine 2014;32:6847-54.
9. Rynkiewicz D, Rathkopf M, Sim I, et al. Marked enhancement of the immune response to BioThrax(R) (Anthrax Vaccine Adsorbed) by the TLR9 agonist CPG 7909 in healthy volunteers. Vaccine 2011;29:6313-20.
10. Galena HJ. Complications occurring from diagnostic venipuncture. J Fam Pract 1992;34:582-4.
11. Ramos JA. Venipuncture-related lateral antebrachial cutaneous nerve injury: what to know? Braz J Anesthesiol 2014;64:131-3.
12. Atanasoff S, Ryan T, Lightfoot R, Johann-Liang R. Shoulder injury related to vaccine administration (SIRVA). Vaccine 2010;28:8049-52.
13. Hopkins RJ, Kalsi G, Montalvo-Lugo VM, et al. Randomized, double-blind, active-controlled study evaluating the safety and immunogenicity of three vaccination schedules and two dose levels of AV7909 vaccine for anthrax post-exposure prophylaxis in healthy adults. Vaccine 2016;34:2096-105.
14. Segal BM, Chang JT, Shevach EM. CpG oligonucleotides are potent adjuvants for the activation of autoreactive encephalitogenic T cells in vivo. J Immunol 2000;164:5683-8.
15. Cooper CL, Angel JB, Seguin I, Davis HL, Cameron DW. CPG 7909 adjuvant plus hepatitis B virus vaccination in HIV-infected adults achieves long-term seroprotection for up to 5 years. Clin Infect Dis 2008;46:1310-4.

16. Cooper CL, Davis HL, Angel JB, et al. CPG 7909 adjuvant improves hepatitis B virus vaccine seroprotection in antiretroviral-treated HIV-infected adults. AIDS 2005;19:1473-9.
17. Ellis RD, Martin LB, Shaffer D, et al. Phase 1 trial of the Plasmodium falciparum blood stage vaccine MSP1(42)-C1/Alhydrogel with and without CPG 7909 in malaria naive adults. PLoS One 2010;5:e8787.
18. Mullen GE, Ellis RD, Miura K, et al. Phase 1 trial of AMA1-C1/Alhydrogel plus CPG 7909: an asexual blood-stage vaccine for Plasmodium falciparum malaria. PLoS One 2008;3:e2940.

# 18 APPENDICES

**Appendix A:** Schedule of Study Procedures and Evaluations

**Appendix B**: Adverse Events of Special Interest

# APPENDIX A. SCHEDULE OF STUDY PROCEDURES AND EVALUATIONS

| **Study Visit (V)** | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **Early Termination** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Study Day[#]** | **pre** | **1** | **8** | **15[#]** | **22[#]** | **29[#]** | **64[#]** | **195[#]** | **380[#]** | |
| **Window Open** | **-28** | **1** | **8** | **15** | **22** | **27** | **60** | **188** | **373** | |
| **Window Close** | **-2** | **1** | **11** | **18** | **25** | **31** | **68** | **202** | **387** | |
| **Study Week** | **pre** | **1** | **1** | **2** | **3** | **4** | **9** | **28** | **54** | |
| **Activity/Procedure** | | | | | | | | | | |
| Inform prospective participant about study details[1] | X | | | | | | | | | |
| Obtain informed consent, HIPAA authorization, enroll[2] | X | | | | | | | | | |
| Obtain, record, update demographics, medical history, concomitant medications[3] | X | X | X | X | X | X | X | X | X | X |
| Perform physical examination[4] | X | X | X | X | X | X | X | X | X | X |
| Collect blood/urine for eligibility or post-vaccination safety[5] | X | | | | | X | | | | X |
| Perform pregnancy test[6] | X | X | | X | | | | | | |
| Perform 12-lead electrocardiogram[7] | X | | | | | | | | | |
| Review eligibility criteria or criteria for subsequent vacination[8] | X | X | | X | | | | | | |
| Perform randomization[9] | | X | | | | | | | | |
| Vaccinate[10] | | X | | X | | | | | | |
| Perform post-vaccination reactogenicity assessment, instruct on eMemory Aid, & provide study related materials[11] | | X | | X | | | | | | |
| Counsel on avoidance of pregnancy[12] | X | X | X | X | X | X | X | | | |
| Review memory aid (solicited AEs/reactogenicity) & confirm data[13] | | | X | | X | | | | | |
| Examine study vaccination site[14] | | | X | | X | | | | | X |
| Collect blood – Toxin Neutralization Assay (TNA) and anti-PA IgG by ELISA[15] | | X | X | X | X | X | X | X | X | X |
| Collect blood – to be stored for use if PIMMC occurs[16] | X | | | | | | | | | |
| Perform assessment[17] of non-serious unsolicited AEs | | X | X | X | X | X | X | | | X |
| Perform SAE, MAAE, and AESI assessment[18] | | X | X | X | X | X | X | X | X | X |

[#] Note that all visits after Visit 3 (target Day 15, second vaccination) are based on the day that Visit 3 occurs. If it occurs on Day 15, all subsequent visits and windows are as written in Appendix A. If it occurs after Day 15, subsequent visit days will be adjusted to maintain the intervals. For example, if Visit 3 occurs on Day 16, then the remaining target Days are 23, 30, 65, 196, and 381. The number of days in the accompanying acceptable windows for each visit will remain the same. The Days for each visit, as written in the Table above and described throughout the protocol and elsewhere, reflect the schedule assuming that Visit 3 occurs on Day 15.

1. Prior to screening, participants may be given information about the study design and purpose, eligibility criteria, procedures, and other approved details by telephone or otherwise. At the screening visit, prospective participants will be provided detailed information about the trial, will be asked to review the informed consent document, will have all their questions answered, and will be evaluated for their ability to provide informed consent.

2. Participants will provide written informed consent, including optional secondary use provisions, HIPAA authorization, acknowledgment of the Notice of Privacy Practices, and other study research related documents. Upon completion of the initial informed consent process, they will be enrolled and will begin screening.
3. In a private space, trained study team members will collect demographics, medical history, and concomitant medications. At screening and the Day 1 visit, these activities will be performed for the purposes of determining eligibility and baseline health status. On subsequent visits, updates to the medical history and medications will be collected and recorded to evaluate for AEs. Concomitant medications will be recorded routinely through Day 64. After Day 64, only concomitant medications (new and updates to previously reported ones) that are taken in relation to MAAEs, SAEs, and AESIs (PIMMCs) will be recorded.
4. At screening, a more detailed physical examination will be performed to determine eligibility and any baseline findings. This exam includes a height and weight for the determination of Body Mass Index (BMI). At Day 1 and at subsequent visits, a physical examination will only be performed if the participant reports a symptom warranting an exam. The screening exam will be "detailed"; the subsequent exams, when needed, will be "targeted". The screening exam, Day 1, Day 15, and ET visits will also include vital signs (blood pressure, heart rate, oral temperature). Vital signs may be repeated at the discretion of the clinician, up to 3 times.
5. Blood and urine will be collected at screening to determine eligibility. Blood will be collected at Day 29 to determine laboratory safety. Tests performed are found in the protocol.
6. A serum pregnancy test (β hCG) will be sent with the screening laboratory specimens and a point-of-care urine pregnancy test will be performed on Days 1 and 15, with results determined to be negative before randomization and vaccination. Should Visit 3 (second vaccination) occur after Day 15, the pregnancy test will be performed on the day of the visit, with results determined to be negative before vaccination.
7. A routine 12-lead electrocardiogram will be performed at screening. It will be read by the automated ECG machine, reviewed by the investigator, and officially read by a cardiologist. Participants with clinically significant abnormalities will be excluded. Adjustments may be made while the ECG is being performed, but once it is complete, no repeat ECGs will be performed unless the reviewing cardiologist requires a repeat study for clarification. No repeat ECGs will be performed after vaccination.
8. Prior to randomization and enrollment, the PI or his/her delegate will review all eligibility criteria and record his/her determination. Prior to the second dose on Day 15 (or as late as Day 18), the PI or his/her delegate will review all criteria for subsequent dosing and record his/her determination.
9. Upon determination of eligibility, the pharmacy will be notified to proceed with randomization and preparation of the vaccine either as the liquid formulation or lyophilized formulation.
10. A pre-administration reactogenicity assessments will be performed immediately prior to study vaccination to establish baseline. The participant will be vaccinated intramuscularly, in the deltoid of the arm chosen by the participant, by an unblinded study team member.
11. In the 30 minutes after vaccination, the participant will be evaluated for immediate reactogenicity and will be instructed on the use of the electronic memory aid to record solicited adverse events (reactogenicity) on Days 1 through 8 and Days 15 through 22, inclusive. Should Visit 3 (second vaccination) occur after Day 15, the reactogenicity period will be adjusted to collect a full 7 days of data. Any other study-related materials available for the participant, such as study contact information, ruler, and thermometer, will be provided.
12. Women of childbearing potential will be counseled to avoid pregnancy until 60 days after the second (final) vaccination, i.e., Day 75 if Visit 3 occurs on Day 15.
13. In the 7 days following vaccination (Days 1 through 8, inclusively and Days 15 through 22, inclusively; or adjusted, as described above, should second vaccination occur after Day 15), during which the participants will be directly entering their reactogenicity profiles through a secure web portal, the study team will be reviewing the entries, answering questions, and contacting the participant if they have failed to enter data. On Days 8 and 22 (or 7 days after the second vaccination, if the second vaccination occurs after Day 15), when the post-vaccination diaries will have been completed, the study team will confirm all entries. At these visits, the participant will also be asked about unsolicited AEs. If solicited AEs persist in Day 8 or Day 22 (beyond the seventh day after second vaccination), they will be followed to resolution.
14. The site of the vaccination will be examined and findings recorded on the appropriate CRF.

15. The toxin neutralization assay is the gold standard immunologic assay for protection against anthrax. It is performed on serum. ELISA will also be performed, to measure the concentration of anti-PA IgG. On vaccination days (Visits 1 and 3), these will be collected prior to vaccination.
16. Blood will be collected for storage in case the participant develops signs and/or symptoms of a PIMMC.
17. Information on unsolicited AEs that are not serious will be collected from the time of the first study vaccination to the Day 64 visit (or the Day as determined in an adjusted schedule, should the second vaccination, Visit 3, occur after Day 15).
18. Information on SAEs, MAAEs, and AESIs will be collected from the time of the first study vaccination to the Day 380 visit (which may be adjusted, should the second vaccination, Visit 3, occur after Day 15). The only AESIs for this study are potentially immune mediated diseases (PIMMCs). If an AESI is reported, the participant will be asked to have an evaluation performed.

# APPENDIX B. ADVERSE EVENTS OF SPECIAL INTEREST

## PIMMCs:

### Gastrointestinal disorders

- Celiac disease
- Crohn's disease
- Ulcerative colitis
- Ulcerative proctitis

### Liver disorders

- Autoimmune cholangitis
- Autoimmune hepatitis
- Primary biliary cirrhosis
- Primary sclerosing cholangitis

### Metabolic diseases

- Addison's disease
- Autoimmune thyroiditis (including Hashimoto thyroiditis)
- Diabetes mellitus type I
- Grave's or Basedow's disease

### Musculoskeletal disorders

- Antisynthetase syndrome
- Dermatomyositis
- Juvenile chronic arthritis (including Still's disease)
- Mixed connective tissue disorder
- Polymyalgia rheumatic
- Polymyositis
- Psoriatic arthropathy
- Relapsing polychondritis
- Rheumatoid arthritis
- Scleroderma, including diffuse systemic form and CREST syndrome
- Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's Syndrome) and undifferentiated spondyloarthritis
- Systemic lupus erythematosus
- Systemic sclerosis

### Neuroinflammatory disorders

- Acute disseminated encephalomyelitis, including site-specific variants (e.g., non-infectious encephalitis, encephalomyelitis, myelitis, myeloradiculomyelitis)
- Cranial nerve disorders, including paralyses/paresis (e.g., Bell's palsy)
- Guillain-Barré syndrome, including Miller Fisher syndrome and other variants
- Immune-mediated peripheral neuropathies and plexopathies, including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy
- Multiple sclerosis
- Narcolepsy
- Optic neuritis
- Transverse myelitis
- Myasthenia gravis, including Eaton-Lambert syndrome

**Skin disorders**

- Alopecia areata
- Autoimmune bullous skin diseases, including pemphigus, pemphigoid and dermatitis herpetiformis
- Cutaneous lupus erythematosus
- Erythema nodosum
- Morphoea
- Lichen planus
- Psoriasis
- Sweet's syndrome
- Vitiligo

**Vasculitides**

- Large vessels vasculitis including: giant cell arteritis such as Takayasu's arteritis and temporal arteritis
- Medium sized and/or small vessels vasculitis including: polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, Churg-Strauss syndrome (allergic granulomatous angiitis), Buerger's disease thromboangiitis obliterans, necrotizing vasculitis and anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Henoch- Schonlein purpura, Behcet's syndrome, leukocytoclastic vasculitis

**Others**

- Antiphospholipid syndrome
- Autoimmune hemolytic anemia

- Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis)
- Autoimmune myocarditis/cardiomyopathy
- Autoimmune parotitis
- Autoimmune thrombocytopenia
- Good pasture syndrome
- Idiopathic pulmonary fibrosis
- Pernicious anemia
- Raynaud's phenomenon
- Sarcoidosis
- Sjögren's syndrome
- Stevens-Johnson syndrome
- Uveitis