CLINICAL STUDY PROTOCOL

Randomized, Active-Comparison, Double-Blind, Phase 2 Study to Assess the Safety and Immunogenicity of Anthrax Vaccine Adsorbed (BioThrax®) without and with CPG 7909 Adjuvant (AV7909 Anthrax Vaccine), Using a Post-Exposure Prophylaxis Dosing Regimen in Adults 66 Years of Age or Older in Stable Health in Comparison to Adults 18-50 Years of Age in Stable Health

BARDA **S**ecuring **A**nthrax Immunity **F**or the **E**lderly (B-SAFE)

BP-C-17001

Sponsor: Biomedical Advanced Research and Development Authority (BARDA)

[redacted]

Sponsor Contact: [redacted]

Medical Monitor: [redacted]

Version of Protocol: 2.0

Date of Protocol: 16 April 2018

Previous Date and Version: 20 February 2018; Version 1.0

CONFIDENTIAL

All financial and nonfinancial support for this study will be provided by BARDA. The concepts and information contained in this document or generated during the study are considered proprietary and may not be disclosed in whole or in part without the expressed, written consent of BARDA. The study will be conducted according to the Integrated Addendum to International Conference on Harmonisation (ICH) E6 (R1): Guideline for Good Clinical Practice (GCP) E6 (R2).

Protocol Approval - Sponsor Signatory

Study Title	Randomized, Active-Comparison, Double-Blind, Phase 2 Study to Assess the Safety and Immunogenicity of Anthrax Vaccine Adsorbed (BioThrax®) without and with CPG 7909 Adjuvant (AV7909 Anthrax Vaccine), Using a Post-Exposure Prophylaxis Dosing Regimen in Adults 66 Years of Age or Older in Stable Health in Comparison to Adults 18-50 Years of Age in Stable Health
Short Title	**B**ARDA **S**ecuring **A**nthrax Immunity **F**or the **E**lderly (B-SAFE)
Protocol Number	BP-C-17001
Protocol Version	Version 2.0
Protocol Date	16 April 2018

Protocol accepted and approved by:

[redacted]

Signature

4-17-18

Date

Investigator's Agreement

Study Title	Randomized, Active-Comparison, Double-Blind, Phase 2 Study to Assess the Safety and Immunogenicity of Anthrax Vaccine Adsorbed (BioThrax®) without and with CPG 7909 Adjuvant (AV7909 Anthrax Vaccine), Using a Post-Exposure Prophylaxis Dosing Regimen in Adults 66 Years of Age or Older in Stable Health in Comparison to Adults 18-50 Years of Age in Stable Health
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I have read and understood all sections of the above referenced protocol, the current package insert for BioThrax, and the investigator's brochure for AV7909 Anthrax Vaccine.

I agree to supervise all aspects of the protocol at my clinical research site and to conduct the clinical investigation in accordance with the protocol and the International Conference on Harmonisation (ICH) regulations and US IND regulations in 21 CFR 312. I will not make changes to the protocol before consulting with BARDA or implement protocol changes without institutional review board approval except to eliminate an immediate risk to subjects. I agree to administer study treatment only to subjects under my personal supervision or the supervision of a subinvestigator.

I will not supply the investigational product to any person not authorized to receive it. Confidentiality will be protected. Subject identity will not be disclosed to third parties or appear in any study reports or publications.

I will not disclose information regarding this clinical investigation or publish results of the investigation without authorization from BARDA.

_____

Printed Name of Investigator

_____ _____

Signature of Investigator Date

Emergency Contact Information

Table 1: Emergency Contact Information

Role in Study	Name	Address and Telephone Number
Sponsor Contact	[redacted]	[redacted]
Medical Monitor	[redacted]	[redacted]

1. SYNOPSIS

Name of Sponsor/Company: Biomedical Advanced Research and Development Authority (BARDA)	
Name of Investigational Product: BioThrax and AV7909 Anthrax Vaccine (AV7909)	
Name of Active Ingredients: Antigen: Anthrax Vaccine Adsorbed (AVA) Adjuvant: cytosine-guanine dinucleotide (CPG) 7909	
Title of Study: Randomized, Active-Comparison, Double-Blind, Phase 2 Study to Assess the Safety and Immunogenicity of Anthrax Vaccine Adsorbed (BioThrax®) without and with CPG 7909 Adjuvant (AV7909 Anthrax Vaccine), Using a Post-Exposure Prophylaxis Dosing Regimen in Adults 66 Years of Age or Older in Stable Health in Comparison to Adults 18-50 Years of Age in Stable Health	
Short Title: BARDA Securing Anthrax Immunity For the Elderly (B-SAFE)	
Protocol Number: BP-C-17001	
Version Number: 2.0	
Study center(s): Approximately 4 clinical sites in the United States (US)	
Study period (years): Estimated date first subject enrolled: Quarter (Q)2 2018 Estimated date last subject completed: Q4 2019	**Phase of development:** 2

Study Objectives:

Primary Objectives

Safety

- To evaluate safety and reactogenicity, as determined by solicited local and systemic reactogenicity symptoms (within 8 days after each vaccination, inclusive of the vaccination day, excluding reactogenicity on the contralateral arm), of BioThrax or AV7909 administered to adults ≥66 years of age.

Immunogenicity

- To evaluate the seroprotection, defined as toxin neutralization antibody (TNA) 50% neutralization factor (NF_{50}) ≥0.56, rate at Day 64 for BioThrax or AV7909 administered to adults ≥66 years of age.

Secondary Objectives

Safety

- To evaluate the occurrence of treatment-emergent unsolicited adverse events (AEs) (defined as all AEs other than solicited local and systemic reactogenicity symptoms), serious adverse events (SAEs), and medically attended adverse events (MAAEs), including potentially immune-mediated medical conditions (PIMMCs) from the time of the first dose of study investigational product (IP) through 12 months following the last dose of study IP.
- To evaluate the occurrence of solicited local reactogenicity symptoms on the contralateral arm (within 8 days after each vaccination, inclusive of the vaccination day).

Immunogenicity

- To assess the TNA NF_{50} antibody levels, seroprotection rates, and seroconversion (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) rates for each study group at each applicable timepoint through Day 394.
- To assess the TNA effective dilution resulting in 50% neutralization (ED_{50}) antibody levels and seroconversion rates for each study group at each applicable timepoint through Day 394.
- To assess the enzyme-linked immunosorbent assay (ELISA) anti-protective antigen (PA) immunoglobulin G (IgG) antibody levels and seroconversion rates for each study group at each applicable timepoint through Day 394.
- To compare the TNA NF_{50} antibody levels, seroprotection rates, and seroconversion rates through Day 394 between younger adults (18 through 50 years) and older adults (≥66 years of age) within the following groups at each applicable timepoint:
    1. BioThrax given at Days 1, 15, and 29
    2. AV7909 given at Days 1 and 15 and placebo given at Day 29
- To compare the TNA ED_{50} antibody levels and seroconversion rates through Day 394 between younger adults (18 through 50 years) and older adults (≥66 years of age) within the following groups at each applicable timepoint:
    1. BioThrax given at Days 1, 15, and 29
    2. AV7909 given at Days 1 and 15 and placebo given at Day 29
- To compare the ELISA anti-PA IgG antibody levels and seroconversion rates through Day 394 between younger adults (18 through 50 years) and older adults (≥66 years of age) within the following groups at each applicable timepoint:
    1. BioThrax given at Days 1, 15, and 29
    2. AV7909 given at Days 1 and 15 and placebo given at Day 29

Methodology:

After screening, subjects meeting all of the inclusion criteria and none of the exclusion criteria will be randomized to receive either BioThrax or AV7909. Randomization will be stratified by sex and age (18-50, 66-74, and ≥75 years). Subjects ≥66 years of age will be randomized 1:1:1:1 within stratum across 4 treatment arms (approximately 50 subjects per group), and subjects 18 through 50 years of age will be randomized 1:1 within stratum across 2 treatment arms (approximately 50 subjects per group). Once randomized, subjects will receive 3 vaccinations, each separated by approximately 14 days, on Day 1, Day 15, and Day 29, based on their assigned treatment arm.

Immunogenicity assessments will include TNA NF_{50}, TNA ED_{50}, and ELISA anti-PA IgG antibody levels.

Safety assessments will be based on solicited AEs (local and systemic reactogenicity symptoms) occurring within 8 days of each vaccination, treatment-emergent unsolicited AEs (all occurring through Day 50 and only those defined as SAEs, MAAEs, and PIMMCs following Day 50), and treatment-emergent SAEs, MAAEs, and PIMMCs throughout the study. Safety assessments will also include physical examination, vital signs, standard 12-lead electrocardiogram (ECG), high-sensitivity C-reactive protein (hs-CRP) assay, autoantibody assays (if needed), and clinical laboratory tests (chemistry, hematology, and urinalysis). Safety for each subject will be assessed from the time of the first dose of IP through 12 months following the last dose of IP.

Number of subjects (planned):

The proposed enrollment for this study is approximately 300 subjects in stable health (200 subjects aged ≥66 years and 100 subjects aged 18 through 50 years, inclusive). Although not required, ideally ≥1/3 of the elderly subjects enrolled would be in the ≥75 years of age stratum.

There will be 6 study groups (approximately 50 subjects per group).

Study Group	Age Group	Arm	Investigational Product	Dose 1 Day 1	Dose 2 Day 15	Dose 3 Day 29
Group 1	Age ≥66	1	BioThrax	BioThrax	BioThrax	BioThrax
Group 2	Age ≥66	2	AV7909	AV7909	AV7909	AV7909
Group 3	Age ≥66	3	AV7909	AV7909	AV7909	Placebo
Group 4	Age ≥66	4	AV7909	AV7909	Placebo	AV7909
Group 5	Age 18-50	1	BioThrax	BioThrax	BioThrax	BioThrax
Group 6	Age 18-50	3	AV7909	AV7909	AV7909	Placebo

Diagnosis and main criteria for inclusion:

Subjects will be randomized to study treatment only if they meet all of the applicable inclusion criteria and none of the exclusion criteria. In addition, applicable eligibility criteria must be reviewed just prior to each vaccination; if the subject no longer meets applicable eligibility criteria, the investigator, in consultation with the medical monitor in cases of uncertainty, must determine whether the subject should receive the IP dose or be terminated early from study IP.

Deviations from the inclusion and exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability, or subject safety. Therefore, adherence to the criteria specified in the protocol is essential.

Subject Inclusion Criteria

1. Male or nonpregnant females, 66 years of age or older at the time of randomization for the elderly population, or 18 through 50 years of age at the time of randomization for the younger population.
2. Females who are of childbearing potential and are sexually active with a male partner must agree to use an acceptable method of birth control from Screening to Day 64 and must have used a reliable birth control method for at least 2 months prior to Screening.
    a. A female of childbearing potential is defined as post onset menarche and pre-menopausal female capable of becoming pregnant. This does not include females who meet any of the following conditions: menopausal >2 years, tubal ligation >1 year, bilateral salpingo-oophorectomy, or hysterectomy.
    b. Adequate contraception is defined as a contraceptive method with a failure rate of less than 1% per year when used consistently and correctly and when applicable, in accordance with the product label. Examples include: oral contraceptives, either combined or progestogen alone; injectable progestogen; implants of etenogestrel or levonorgestrel; estrogenic vaginal ring; percutaneous contraceptive patches; intrauterine device or intrauterine system; male partner sterilization at least 6 months prior to the female subject's Screening Visit, and this male is the sole partner for that subject (the information on the male partner's sterility can come from the site personnel's review of the subject's medical records or interview with the subject on her medical history); male

condom combined with a vaginal spermicide (foam, gel, film, cream, or suppository); male condom combined with a female diaphragm, either with or without a vaginal spermicide (foam, gel, film, cream, or suppository).

3. Able to provide written informed consent prior to initiation of any study procedures. As part of the consent process, subjects must be able to demonstrate understanding by passing the "Assessment of Understanding Questionnaire" within 2 attempts. Passing is defined as being able to answer all questions correctly.
4. In relatively stable health based on site investigator's judgment, as determined by medical history, physical examination, and the following criteria:
    a. Stable health for age (defined as no new conditions per medical history, new medications in a different therapeutic class, or change in daily dose of existing prescription medications within the 45 days preceding Screening). Effective treatment (to resolution) of an acute infection (e.g., urinary tract infection, cellulitis, otitis, or bronchitis) with an antibiotic within 45 days preceding Screening will not be considered a deviation from this inclusion criterion as long as the antibiotic therapy was completed at least one week prior to Screening and no signs or symptoms of the infection have been present since the completion of treatment. Any prescription change that is due to change of health care provider or insurance company, or that is made for reasons that do not reflect a change in disease status (e.g., financial considerations), as long as within the same general class of medication, will not be considered a deviation from this inclusion criterion. Any change in prescription medication due to improvement of a disease outcome, as determined by the site investigator, will not be considered a deviation from this inclusion criterion.
    b. Subjects may be on chronic or as needed medications if, in the opinion of the site investigator, these pose no additional risk to subject safety or assessment of reactogenicity and immunogenicity and their use is not for management of a worsening of medical diagnosis or condition. Note: Topical, nasal, and inhaled medications (with the exception of steroids, as outlined in the subject exclusion criteria) and vitamins are permitted.
5. Have a body mass index (BMI) less than 35.0 kg/m^2 at Screening. (BMI will not be reassessed prior to subsequent vaccinations.)
6. Have access to a consistent and reliable means of telephone contact, which may be home, workplace, or by personal mobile electronic device.
7. Are available and can comply with all study visits.

Subject Exclusion Criteria

1. Females who have a positive urine pregnancy test at Screening or within 24 hours prior to each study vaccination or women who are breastfeeding.
2. Have a requirement for skilled nursing care.
3. Have a history of severe reactions to components of AVA or CPG 7909, e.g. formaldehyde, benzethonium chloride (phemerol), or aluminum.
4. Have a history of latex sensitivity.
5. Have a history of ever receiving a vaccine for anthrax prior to Screening, or had an anthrax infection.
6. Have any medical, psychiatric, or social condition that, in the opinion of the site investigator, unfavorably alters the risk-benefit of subject participation, or is likely to interfere with study compliance or assessments, or is likely to confound interpretation of safety or immunogenicity data.

7. Have any diagnosis, current or past, of a potentially immune-mediated medical condition, such as Guillain-Barré syndrome, narcolepsy, or an autoimmune or chronic inflammatory disease.
8. Have any diagnosis, current or past, of schizophrenia or bipolar disorder.
9. Have been hospitalized for psychiatric illness, have a history of suicide attempt, or confinement for danger to self or others within the preceding 10 years prior to Screening and each study vaccination.
10. Have a history of alcohol or drug abuse (per investigator's judgment) within 5 years prior to Screening and each study vaccination, or test positive for drugs of abuse at Screening (including for cannabis, even where legal). Benzodiazepines for anxiety disorders and stimulants for attention deficit hyperactivity disorder are not exclusionary if the subject has been on a stable dose for more than 3 months prior to Screening and each study vaccination and if the subject can produce a valid, current prescription for the medication. Propoxyphene, opioids, or combinations containing these medications (including as used for opioid addiction) are not permitted regardless of prescription status. Note: A positive Screening urine drug screen may not be repeated. (Drug screen will not be repeated prior to subsequent vaccinations.)
11. Have known human immunodeficiency virus (HIV), or acute or chronic hepatitis B or hepatitis C infection based on medical history; or test positive for any of these at Screening. Subjects who have been effectively treated for hepatitis C, as evidenced by a negative hepatitis C ribonucleic acid (RNA) confirmation test and who no longer require antiviral therapy, are eligible for participation. (Screening tests will not be repeated prior to subsequent vaccinations.)
12. Have known active neoplastic disease or a history of any hematologic malignancy. However, subjects with superficial skin cancer who do not require intervention other than local excision are not excluded.
13. Have immunosuppression as a result of an underlying illness or treatment, or use of anticancer chemotherapy (cytotoxic) or radiation therapy within 3 years prior to Screening and each study vaccination.
14. Have taken any systemic immunosuppressive agents, including immunomodulators, 6 months prior to Screening and each study vaccination. Allergen immunotherapy (desensitization) is not exclusionary. Allergen immunotherapy (desensitization) does not include systemic antibodies such as benralizumab, mepolizumab, or omalizumab; these are exclusionary.
15. Are suffering from or have a history of neuralgia, paresthesia, or neuritis within 90 days prior to Screening and each study vaccination; or have any history of stroke.
16. Have had convulsions or encephalomyelitis within three years prior to Screening and each study vaccination.
17. Have received immunoglobulin or other blood products (with the exception of Rho[D] immune globulin) within 90 days prior to Screening and each study vaccination.
18. Have received an experimental agent within 180 days prior to the first study vaccination, or expects to participate in another clinical trial with an interventional agent during the study period. This includes licensed or unlicensed vaccines, drugs, biologics, devices, blood products, or medications. Participation in an observational study is not exclusionary as long as it doesn't interfere with study visits or procedures (e.g., blood collection volume restrictions).
19. Have taken oral or parenteral corticosteroids of any dose within 30 days prior to each study vaccination.
20. Have taken high-dose inhaled corticosteroids within 30 days prior to each study vaccination. High-dose is defined as >800 mcg/day of beclomethasone dipropionate or equivalent. Lower doses of inhaled corticosteroids are not exclusionary.
21. Have received any licensed live vaccine within 30 days prior to the first study vaccination or

planned receipt from the first study vaccination through Visit 10 (Day 64).

22. Have received any licensed inactivated vaccine within 14 days prior to the first study vaccination or planned receipt from the first study vaccination through Visit 10 (Day 64).
23. Have an acute illness, as determined by the site investigator, within 72 hours prior to each study vaccination.
    a. If subject's oral temperature is above 100.0°F (37.8°C), the subject may be re-assessed for eligibility within the visit window (+3 Days).
    b. An acute illness that is nearly resolved, with only minor residual symptoms remaining, is allowable if, in the opinion of the site investigator, the residual symptoms will not interfere with the ability of study staff to assess safety parameters as required by the protocol.
    c. For bacterial infections that have been successfully treated with antibiotics, inclusion criterion 4a applies.
24. Have a history of myocardial infarction or ischemia, or have ECG findings of myocardial infarction, ischemia, strain, complete bundle branch block, or ventricular arrhythmias (other than unifocal premature ventricular contractions ≤2/minute). Additionally, other cardiac history or ECG findings, which in the opinion of the investigator represent a potentially unstable or unacceptably high risk of an adverse cardiac outcome, are exclusionary. (ECG will not be repeated prior to subsequent vaccinations.)
25. Have any laboratory test result or clinical findings (including vital signs) that singly or in combination are, in the investigator's opinion, likely to unfavorably alter the risk-benefit of subject participation or to confound study safety or immunogenicity results. Additionally, the following are exclusionary:
    a. Any clinically significant Grade 3 laboratory or vital sign result, or any Grade 4 laboratory or vital sign result (regardless of assessed significance) at Screening. For subsequent vaccinations, abnormal laboratory results and vital sign results post-vaccination will be assessed against the rules for early termination of dosing.
    b. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >2 times the upper limit of normal (ULN), or bilirubin >1.5 times the ULN unless isolated Gilbert's syndrome.
    c. Creatinine >1.5 times ULN for age and sex.
    d. White blood cell count <3,000/µL or >12,500/µL; absolute neutrophil count <1200/µL; absolute lymphocyte count <750/µL; hemoglobin <10 g/dL females, or <11.5 g/dL males; platelet count <75,000/µL.
    e. Hemoglobin A1C (HbA1C) >7.0% at Screening. (HbA1C will not be repeated prior to subsequent vaccinations.)

Subjects cannot be rescreened for exclusionary laboratory test results. Potentially exclusionary vital sign results may be repeated, but unless they have resolved to a Grade 3 designation or less *and* are not considered clinically significant, the subject should be excluded. The most recent laboratory and vital sign results will be used when determining eligibility for subsequent vaccinations.

Investigational product, dosage and mode of administration: Subjects will receive either 3 doses of active IP (BioThrax or AV7909) or 2 doses of active IP (AV7909) and 1 dose of placebo IP. BioThrax (0.5 mL) should be administered by subcutaneous (SC) injection over the deltoid muscle. AV7909 (0.5 mL) should be administered by intramuscular (IM) injection in the deltoid muscle. Placebo IP (0.5 mL) will be administered IM in the deltoid muscle.

Duration of treatment: Subjects will receive 3 study vaccinations, separated by approximately 14 days,

and will be followed for 12 months after their last IP dose. The expected study duration is approximately 14 months per subject.

Reference therapy, dosage and mode of administration: Not applicable

Criteria for evaluation:

Primary Endpoints

There are 2 primary endpoints for this study.

- All solicited local and systemic reactogenicity symptoms occurring within 8 days after each vaccination, inclusive of the vaccination day, excluding reactogenicity symptoms on the contralateral arm.
- Seroprotection at Day 64, defined as a TNA NF_{50} antibody level $\geq$0.56.

Secondary Endpoints

Secondary Safety Endpoints

- All treatment-emergent unsolicited AEs.
- All treatment-emergent SAEs occurring during study participation.
- All treatment-emergent MAAEs occurring during study participation.
- All treatment-emergent PIMMCs occurring during study participation.
- All solicited local reactogenicity symptoms on the contralateral arm occurring within 8 days after each vaccination, inclusive of the vaccination day.

Secondary Immunogenicity Endpoints

- TNA NF_{50} antibody levels at Days 1, 8, 22, 29, 36, 43, 50, 64, 85, 181, and 394.
- TNA ED_{50} antibody levels at Days 1, 8, 22, 29, 36, 43, 50, 64, 85, 181, and 394.
- ELISA anti-PA IgG antibody levels at Days 1, 8, 22, 29, 36, 43, 50, 64, 85, 181, and 394.
- Seroprotection based on TNA NF_{50} at Days 1, 8, 22, 29, 36, 43, 50, 85, 181, and 394.
- Seroconversion based on TNA NF_{50} at Days 8, 22, 29, 36, 43, 50, 64, 85, 181, and 394, defined as a $\geq$4-fold increase over baseline levels, or a $\geq$4-fold increase over the lower limit of quantification (LLOQ) if the baseline value is below the LLOQ.
- Seroconversion based on TNA effective dilution resulting in 50% neutralization (ED_{50}) at Days 8, 22, 29, 36, 43, 50, 64, 85, 181, and 394.
- Seroconversion based on ELISA anti-PA IgG at Days 8, 22, 29, 36, 43, 50, 64, 85, 181, and 394.

Statistical methods:

Analysis Plan

Due to the exploratory nature of the primary objectives, inferential analyses are planned only as part of

the secondary analyses. Descriptive statistics (such as medians, quartiles, and ranges for continuous data, and percentages for categorical data) will be used to summarize subject characteristics, safety, and immunogenicity parameters. These summaries will be presented overall and separately for the subjects in the different study groups, as well as for pooled groups by treatment arm (regardless of age group) and by age group (regardless of treatment arm). Details of the statistical analyses, methods, and data conventions will be described in the Statistical Analysis Plan (SAP).

Interim Analysis

An interim analysis will be performed based on cumulative immunogenicity and safety data through Day 64 for all subjects. Data for the interim analysis will be presented solely at the group level, thus, the blinding at the subject level will be maintained for those outside of the unblinded team until database lock of all data through Day 394 for the clinical study report. All primary and secondary endpoint analyses up through Day 64 will be performed for the interim analysis.

Safety Monitoring Committee Reviews

The Safety Monitoring Committee (SMC) will perform a planned review of interim safety data after 40 subjects ≥66 years of age have completed Visit 7 (Day 36). Enrollment and dosing will continue during this planned SMC review. In addition, ad hoc reviews will occur in the event that pausing/stopping rules are met or a review is deemed necessary by the SMC chair.

The final SMC safety displays will be provided to the SMC members when the last study subject completes Visit 10 (Day 64).

Data displays for each SMC review will be generated by an unblinded statistician. Safety analyses for this study will be descriptive rather than inferential. Detailed listings and summary tabulations will be generated as specified in the SMC charter. The safety analyses will be completed using the safety population.

Final Analyses

A clinical study report will be written to include all safety and immunogenicity data through Day 394. Study data will be unblinded to prepare the study report. Further details will be specified in the SAP.

Analysis Populations

Safety Population

The safety population will include all subjects who are randomized and receive at least 1 IP dose. Each subject will be analyzed as part of the study group corresponding to the actual treatment received for the applicable dose for individual dose summaries. For all other analyses by study group, each subject will be analyzed corresponding to the actual treatment received for the first dose. The safety population will be used for all safety analyses.

Immunogenicity Full Analysis Population

The immunogenicity full analysis population (IFAP) will include all subjects who are randomized, receive at least 1 IP dose, and have at least one determinate assay result at any post-vaccination visit. Each subject will be analyzed based on the treatment and dosing schedule actually received, regardless of the treatment arm assignment. The IFAP will be used only for analysis of seroprotection based on TNA NF50 antibody levels at Day 64 as a secondary analysis. Any additional immunogenicity analyses on the IFAP will be defined in the SAP.

Immunogenicity Per Protocol Population

The immunogenicity per protocol population (IPPP) will include all subjects in the IFAP who meet the following criteria:

- Receive a full dose of IP at Day 1, Day 15, and Day 29 within protocol specified visit windows (- 1 Day, +3 Days).
- Receive the correct treatment as assigned by randomization at Day 1, Day 15, and Day 29.
- Have no other major protocol deviations that may have an impact on immunogenicity assessments.
- Have determinate assay results at the Day 64 visit.

The IPPP will be used for all primary and secondary immunogenicity endpoint assessments.

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3. LIST OF ABBREVIATIONS

Abbreviation or Specialist Term	Explanation
AE	adverse event
ALT	alanine aminotransferase
ASPR	Assistant Secretary for Preparedness and Response
AST	aspartate aminotransferase
AVA	Anthrax Vaccine Adsorbed
AV7909	AV7909 Anthrax Vaccine
B. anthracis	*Bacillus anthracis*
BARDA	Biomedical Advanced Research and Development Authority
BMI	body mass index
B-SAFE	BARDA Securing Anthrax Immunity For the Elderly
CFC	chlorofluorocarbon
CFR	Code of Federal Regulations
CI	confidence interval
CPG	cytosine-guanine dinucleotide
DMP	Data Management Plan
eCRF	electronic case report form
ED_{50}	effective dilution resulting in 50% neutralization
ECG	Electrocardiogram
ELISA	enzyme-linked immunosorbent assay
Emergent	Emergent BioSolutions
ET	early termination
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HbA1C	hemoglobin A1C
HHS	Department of Health and Human Services
hs-CRP	high-sensitivity C-reactive protein
ICF	informed consent form
ICH	International Conference on Harmonisation

Abbreviation or Specialist Term	Explanation
IFAP	immunogenicity full analysis population
IgG	immunoglobulin G
IM	Intramuscular
IP	investigational product
IPPP	immunogenicity per protocol population
IRB	institutional review board
IWRS	interactive web response system
kit ID	unique kit number
LLOQ	lower limit of quantification
MAAE	medically attended adverse event
NF_{50}	50% neutralization factor
OTC	over-the-counter
PA	protective antigen
PEP	post-exposure prophylaxis
PIMMC	potentially immune-mediated medical condition
Q	quarter
SAE	serious adverse event
SAP	Statistical Analysis Plan
SAR	suspected adverse reaction
SC	Subcutaneous
SIP	Strategy and Implementation Plan
SMC	Safety Monitoring Committee
SUSAR	serious and unexpected suspected adverse reaction
TEAE	Treatment-emergent adverse event
TNA	toxin neutralization antibody
ULN	upper limit of normal
US	United States

4. INTRODUCTION

4.1. Background

Anthrax is a zoonotic, acute infectious disease caused by the Gram-positive, spore-forming bacterium *Bacillus anthracis* (*B. anthracis*), which can cause human disease via gastrointestinal, cutaneous, inhalational (pulmonary), or injection routes. Although clinical manifestations of the disease differ by route, inhalation anthrax is the most lethal. Inhalation anthrax infection usually develops within a week after exposure, but it can take up to 2 months. Without treatment, only about 10 to 15% of patients with inhalation anthrax survive; with aggressive treatment, however, about 55% of patients survive. Although anthrax is rare in the United States (US), people who handle animal products and members of the US military are at greater risk of exposure. In addition, *B. anthracis* is one of the most likely agents to be used in a bioterrorist attack. In 2001, anthrax spores were put in letters mailed through the US postal system resulting in 22 people infected, from which 5 died. [1]

The mission of the Biomedical Advanced Research and Development Authority (BARDA) within the Department of Health and Human Services (HHS) is to develop and procure medical countermeasures that address the public health and medical consequences of chemical, biological, radiological, and nuclear accidents, incidents, and attacks; pandemic influenza; and emerging infectious diseases. The HHS' involvement in the development and acquisition of anthrax antitoxins and vaccines is coincident with the signing of the Project BioShield Act of 2004.[2] Several medical interventions contribute to mitigating the high morbidity and mortality from inhalation anthrax, including vaccines, antibiotics, and antitoxins, all part of the HHS anthrax medical countermeasures strategy. Since the majority of individuals are not vaccinated against anthrax, strategies to implement post-exposure prophylaxis (PEP) in case of a bioterrorist attack are needed.

The only Food and Drug Administration (FDA)-approved vaccine for anthrax (Anthrax Vaccine Adsorbed [AVA], BioThrax®) is licensed in the US for adults 18 through 65 years of age. BioThrax is made from cell-free filtrates of microaerophilic cultures of an avirulent, nonencapsulated strain of *B. anthracis*, and induces antibodies against protective antigen (PA) that may contribute to protection by neutralizing the activities of the lethal toxin and edema toxin of *B. anthracis*.[3] *B. anthracis* proteins other than PA may be present in BioThrax, but their contribution to protection has not been determined. BioThrax is approved in the US for general use pre-exposure prophylaxis in individuals who are at high risk of infection (e.g. military and lab workers); the current regimen calls for a three-dose priming series (0, 1, and 6 months) with boosters at 12 and 18 months (via the intramuscular [IM] route) after the initial priming series, and annual boosters thereafter. BioThrax is also approved for PEP, which calls for a series of 3 immunizations at 0, 2, and 4 weeks when administered in conjunction with recommended antibacterial drugs.[4] For the PEP indication, BioThrax is administered by the subcutaneous (SC) route.

4.2. Study Rationale

In 2016, the Public Health Emergency Medical Countermeasures Enterprise published its revised Strategy and Implementation Plan (SIP). Of the objectives outlined within the SIP, one primary goal is to address medical countermeasure gaps for all sectors of the US civilian population. Two primary population gaps, pediatric and geriatric, exist for FDA approved/licensed medical countermeasures, including anthrax. In an effort to address these medical countermeasures gaps, BARDA is working to generate data to help inform policy makers on the appropriate use of medical countermeasures in these "at-risk" populations.

Antibody responses to vaccination with BioThrax diminish in progressively older age cohorts up to 65 years of age when compared to younger adults.[5] It is anticipated that further reductions in antibody response to BioThrax will occur in individuals 66 years of age and older and the inclusion of an adjuvant will improve immunogenicity without major increases in acute post-vaccination adverse event rates.

AV7909 Anthrax Vaccine (henceforth AV7909), a combination product containing BioThrax and the adjuvant cytosine-guanine dinucleotide (CPG) 7909, is under development as a potential next generation anthrax vaccine. CPG 7909 is a synthetic immunostimulatory oligodeoxynucleotide that has been shown to be a potent vaccine adjuvant. CPG 7909 is a Toll-like receptor 9 agonist designed to induce both an enhanced antigen-specific antibody response and a natural killer T-cell immune response when used in combination with prophylactic (preventative) or therapeutic vaccines.[6,7,8,9] Earlier studies have suggested that AV7909 vaccine has achieved, compared with BioThrax vaccine, higher serum toxin neutralization antibody (TNA) levels, an accelerated immune response, and fewer injections to confer protection in subjects 18 through 50 years old.[10,11,12] While BioThrax is administered by the subcutaneous route for its PEP indication, AV7909 is being developed for intramuscular administration.

This randomized, phase 2, active-controlled, double-blinded, multi-site study is designed to assess the safety and immunogenicity of BioThrax and AV7909 using a PEP dosing regimen in adults ≥66 years of age in stable health compared to subjects 18 through 50 years of age in stable health. Three different PEP dosing regimens for AV7909 will be explored to determine optimal dosing (Table 2).

4.3. Previous Clinical Studies

4.3.1. BioThrax Post-Exposure Prophylaxis

In 2015, FDA approved BioThrax for PEP in adults 18 through 65 years of age when administered in conjunction with recommended antibacterial drugs.[4] BioThrax became the first vaccine to receive licensure for a new indication based on FDA's Animal Rule.[13] The PEP regimen was approved based on adequate and well-controlled animal studies in rabbits and nonhuman primates demonstrating that serum TNA and anti-PA immunoglobulin G (IgG) titers were reliable predictors of survival following lethal *B. anthracis* challenge. A 70% probability of survival was associated with a TNA 50% neutralization factor (NF_{50}) level of 0.56 in rabbits and 0.29 in nonhuman primates.

Based on the rabbit model-derived TNA threshold, a pivotal clinical study was conducted to evaluate the immunogenicity and safety of a PEP SC administration schedule of BioThrax in healthy adults following 3 doses at 0, 2, and 4 weeks. Two hundred subjects were enrolled and followed for 128 days. The primary objective was to assess immunogenicity using TNA following the completion of 3 SC doses of BioThrax. The primary immunogenicity endpoint was the proportion of subjects achieving a threshold TNA NF_{50} value ≥0.56 at Day 63, 5 weeks after the third vaccination. Success was concluded if the lower bound of the 2-sided 95% confidence interval (CI) of the proportion of human subjects achieving the TNA NF_{50} threshold was ≥40%. Overall, 71.2% of subjects achieved a TNA NF_{50} value ≥0.56 on Day 63 in the pivotal study. The lower bound of the 95% CI was 64.1%.

No studies in subjects ≥66 years old have been performed with BioThrax.

4.3.2. AV7909

Emergent sponsored three clinical studies in healthy subjects 18 through 50 years of age as part of the AV7909 development program including a phase 1, a phase 1/2, and a phase 2 trial.[10,11,12] A total of 241 subjects have been exposed to a combination of AVA and CPG 7909. 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.[12] The second trial identified a formulation of AV7909 with a lower CPG 7909 concentration (0.25 mg CPG 7909) for further clinical development that produced an enhanced immune response without increased reactogenicity.[11] 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.[10] The results of this study found that AV7909 was well tolerated (>90 subjects with adverse events [AEs] of Grade 1 or 2 [based on a modified version of the Guidance for Industry "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (September 2007),[14] henceforth referred to as FDA's standard toxicity grading scales]; 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) and produced a more rapid immune response compared to BioThrax with protective titers of TNA NF_{50} values (TNA ≥0.56) obtained through Day 63.

No studies in subjects ≥66 years old have been performed with AV7909.

5. STUDY OBJECTIVES

5.1. Primary Objectives

5.1.1. Safety

- To evaluate safety and reactogenicity, as determined by solicited local and systemic reactogenicity symptoms (within 8 days after each vaccination, inclusive of the vaccination day, excluding reactogenicity on the contralateral arm), of BioThrax or AV7909 administered to adults ≥66 years of age.

5.1.2. Immunogenicity

- To evaluate the seroprotection, defined as TNA NF_{50} ≥0.56, rate at Day 64 for BioThrax or AV7909 administered to adults ≥66 years of age.

5.2. Secondary Objectives

5.2.1. Safety

- To evaluate the occurrence of treatment-emergent unsolicited AEs (defined as all AEs other than solicited local and systemic reactogenicity symptoms), SAEs, and medically attended adverse events (MAAEs), including potentially immune-mediated medical conditions (PIMMCs) from the time of the first dose of study investigational product (IP) through 12 months following the last dose of study IP.
- To evaluate the occurrence of solicited local reactogenicity symptoms on the contralateral arm (within 8 days after each vaccination, inclusive of the vaccination day)

5.2.2. Immunogenicity

- To assess the TNA NF_{50} antibody levels, seroprotection rates, and seroconversion (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) rates for each study group at each applicable timepoint through Day 394.
- To assess the TNA effective dilution resulting in 50% neutralization (ED_{50}) antibody levels and seroconversion rates for each study group at each applicable timepoint through Day 394.
- To assess the enzyme-linked immunosorbent assay (ELISA) anti-PA IgG antibody levels and seroconversion rates for each study group at each applicable timepoint through Day 394.
- To compare the TNA NF_{50} antibody levels, seroprotection rates, and seroconversion rates through Day 394 between younger adults (18 through 50 years) and older adults (≥66 years of age) within the following groups at each applicable timepoint:
    1. BioThrax given at Days 1, 15, and 29

2. AV7909 given at Days 1 and 15 and placebo given at Day 29

- To compare the TNA ED_{50} antibody levels and seroconversion rates through Day 394 between younger adults (18 through 50 years) and older adults (≥66 years of age) within the following groups at each applicable timepoint:
    1. BioThrax given at Days 1, 15, and 29
    2. AV7909 given at Days 1 and 15 and placebo given at Day 29
- To compare the ELISA anti-PA IgG antibody levels and seroconversion rates through Day 394 between younger adults (18 through 50 years) and older adults (≥66 years of age) within the following groups at each applicable timepoint:
    1. BioThrax given at Days 1, 15, and 29
    2. AV7909 given at Days 1 and 15 and placebo given at Day 29

6. INVESTIGATIONAL PLAN

6.1. Overall Study Design

This is a phase 2, randomized, active-controlled, double-blinded, multi-site study to assess the safety and immunogenicity of BioThrax and AV7909 using a PEP dosing regimen in adults ≥66 years of age in stable health. The safety and immunogenicity profile of BioThrax and AV7909 in adults ≥66 years of age will also be compared to the safety and immunogenicity profile of subjects 18 through 50 years of age in stable health. The main study goal is to determine optimal dosing for AV7909.

Approximately 300 male and nonpregnant female adults (200 aged ≥66 years and 100 aged 18 through 50 years, inclusive) will be enrolled in the study (Section 7.1).

Figure 1 presents a diagram of the overall study design. After screening, subjects meeting all of the inclusion criteria and none of the exclusion criteria will be randomized to receive either BioThrax or AV7909. Randomization will be stratified by sex and age (18-50, 66-74, and ≥75 years). Subjects ≥66 years of age will be randomized 1:1:1:1 within stratum across 4 treatment arms (approximately 50 subjects per group), and subjects 18 through 50 years of age will be randomized 1:1 within stratum across 2 treatment arms (approximately 50 subjects per group) as specified in Table 2. Once randomized, subjects will receive 3 vaccinations, each separated by approximately 14 days, on Day 1, Day 15, and Day 29, based on their assigned treatment arm.

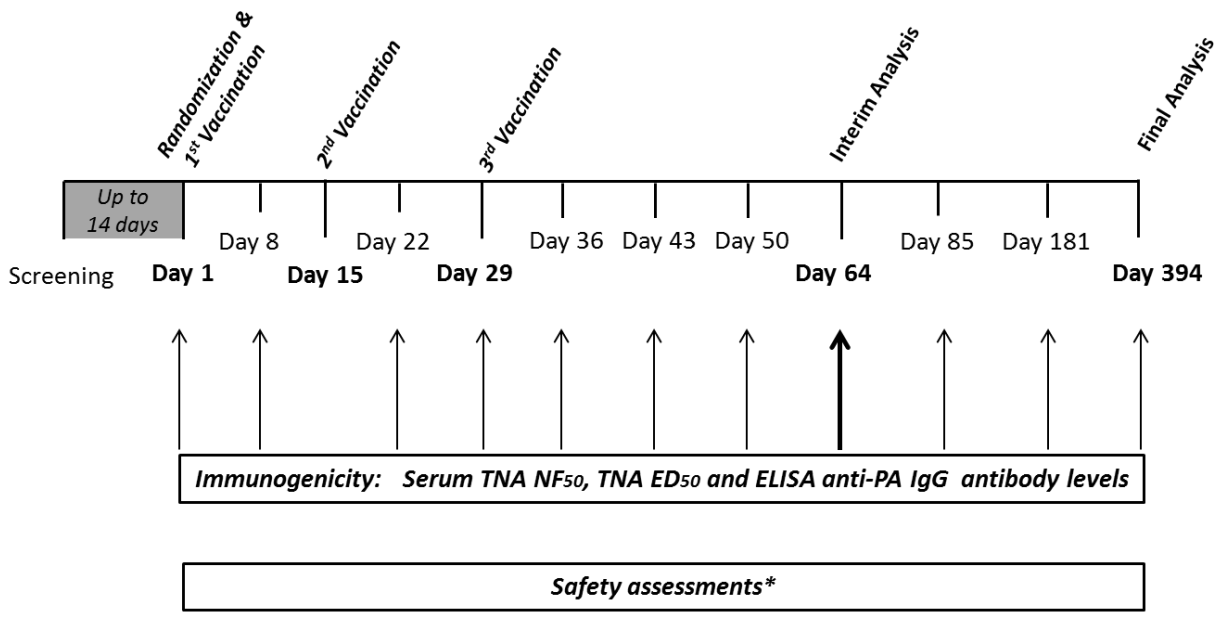
Immunogenicity assessments will include TNA NF_{50}, TNA ED_{50}, and ELISA anti-PA IgG antibody levels. Additional details regarding the immunogenicity assessments are presented in Section 10.

Safety assessments will be based on solicited AEs (local and systemic reactogenicity symptoms) occurring within 8 days of each vaccination, treatment-emergent unsolicited AEs (all occurring through Day 50 and only those defined as SAEs, MAAEs, and PIMMCs following Day 50), and treatment-emergent SAEs, MAAEs, and PIMMCs throughout the study (Figure 1). Safety for each subject will be assessed from the time of the first dose of IP through 12 months following the last dose of IP. Section 11 provides a detailed description of the safety parameters to be assessed during this study.

The expected study duration is approximately 14 months per subject.

The Schedule of Assessments can be found in Appendix 1.

Figure 1: Overall Study Design



*AEs will be assessed from the time the subject receives the first vaccination as follows:

- Solicited AEs (i.e., solicited local and systemic reactogenicity symptoms) will be collected within 8 days after each vaccination, inclusive of the vaccination day (i.e., Day 1 through Day 8, Day 15 through Day 22, and Day 29 through Day 36)
- All unsolicited AEs will be collected from the time the subject receives the first vaccination (Day 1) until Day 50. Following Day 50, unsolicited AEs will only be recorded if they are defined as SAEs, MAAEs, or PIMMCs.
- SAEs, MAAEs and PIMMCs will be assessed from the time the subject receives the first vaccination (Day 1) until exit from the study (Day 394).

AE = adverse event; ED_{50} = effective dilution resulting in 50% neutralization of anthrax lethal toxin; ELISA = enzyme-linked immunosorbent assay; IgG = immunoglobulin G; MAAEs = medically attended adverse events; NF_{50} = 50% neutralization factor; PA = protective antigen; PIMMCs = potentially immune-mediated medical conditions; SAEs = serious adverse events; TNA = toxin neutralization antibody

6.2. Number of Subjects

The proposed enrollment for this study is approximately 300 subjects in stable health (200 subjects aged ≥66 years and 100 subjects aged 18 through 50 years, inclusive). Although not required, ideally ≥1/3 of the elderly subjects enrolled would be in the ≥75 years of age stratum.

6.3. Treatment Assignment

Subjects will receive either 3 doses of active IP (BioThrax or AV7909) or 2 doses of active IP (AV7909) and 1 dose of placebo IP, separated by approximately 14 days, based on their assigned treatment arm. There will be 6 study groups (approximately 50 subjects per group) as presented in Table 2. Randomization will be stratified by sex and age group (18-50, 66-74, and ≥75 years). Subjects ≥66 years of age will be randomized in a 1:1:1:1 ratio within stratum across 4 treatment arms and will compose Groups 1 through 4. Adults aged 18 through 50 years will be randomized in a 1:1 ratio within stratum across 2 treatment arms and will compose Groups 5 and 6.

Table 2: Study Groups

Study Group	Number of Subjects	Age Group	Arm	Investigational Product	Route	Dose 1 Day 1	Dose 2 Day 15	Dose 3 Day 29
Group 1	50	Age ≥66	Arm 1	BioThrax	SC	BioThrax	BioThrax	BioThrax
Group 2	50	Age ≥66	Arm 2	AV7909	IM	AV7909	AV7909	AV7909
Group 3	50	Age ≥66	Arm 3	AV7909	IM	AV7909	AV7909	Placebo
Group 4	50	Age ≥66	Arm 4	AV7909	IM	AV7909	Placebo	AV7909
Group 5	50	Age 18-50	Arm 1	BioThrax	SC	BioThrax	BioThrax	BioThrax
Group 6	50	Age 18-50	Arm 3	AV7909	IM	AV7909	AV7909	Placebo

IM = intramuscular; SC = subcutaneous

6.4. Individual Subject Dosing and Study Stopping Rules

6.4.1. Early Termination of Dosing for an Individual Subject

Vaccinations in a subject are to be stopped if the subject experiences any of the following and will preclude further administration of study vaccine until the specific AE can be evaluated and is shown to resolve completely. In situations of ambiguity or uncertainty, the investigator is encouraged to discuss the situation and any questions with the medical monitor before making a decision on subject disposition.

1. Any Grade 4 AE that is assessed as other than unrelated to the receipt of the IP (Refer to Section 11.4, Table 5, and Appendix 2).
2. Two or more Grade 3 AEs that are assessed as other than unrelated to the receipt of the IP.
3. A clinically significant Grade 3 laboratory value assessed as other than unrelated to IP that remains clinically significant and at Grade 3 or higher upon repeat testing (Refer to Section 11.1.8).
4. Any SAE assessed as other than unrelated to the receipt of the IP.
5. Any PIMMC irrespective of relatedness to IP.
6. Anaphylaxis within 4 hours of vaccination or that is assessed as other than unrelated to IP.
7. Intolerable AE.
8. Pregnancy.
9. Subject no longer meets eligibility criteria in such a way that, in the judgment of the investigator, the safety of the subject may be compromised by continued participation or interpretation of the subject's subsequent study data are likely to be significantly compromised.
10. Any other condition that, in the judgment of the investigator or Safety Monitoring Committee (SMC), would make further vaccination unsafe or render the subject unable to comply with protocol-mandated safety follow-up.

The reason for early termination of dosing will be captured in the electronic case report form (eCRF).

Subjects who do not receive a full vaccination series for any reason will continue to be followed for immunogenicity and safety through Visit 13 (Day 394). Subjects who become pregnant during the study will only be followed for safety and will not provide blood for the immunogenicity assays.

6.4.2. Safety Monitoring Committee

6.4.2.1. Safety Monitoring Committee Reviews

There will be 1 planned SMC review of interim safety data after the first 40 subjects ≥66 years have completed Visit 7 (Day 36). Enrollment and dosing will continue during this planned SMC review.

The SMC chair will receive and evaluate reports of all SAEs and Grade 3 and higher AEs, irrespective of relatedness to IP, on an ongoing basis. Upon review of these reports, the SMC chair will determine whether an ad hoc review by the entire SMC is necessary. Ad hoc reviews will also occur in the event that study pausing/stopping rules are met (Section 6.4.2.2).

In order to preserve the blinded nature of IP assignments during the open session meetings, safety data will be presented overall and not by group assignment. Fully unblinded safety data will be provided by the unblinded statistical team for closed SMC sessions.

Following each review, the SMC will advise the sponsor on their recommendation regarding individual subject disposition or study disposition per guidelines in the SMC Charter. The SMC may recommend continuation of the study with no modifications, continuation of the study with modification, discontinuation of specific study groups, or study termination.

The final SMC safety displays will be provided to the SMC members when the last study subject completes Visit 10 (Day 64).

6.4.2.2. Study Pausing/Stopping Rules

The blinded medical monitor will assess cumulative safety information for this study per the Medical Monitoring Plan and advise BARDA should she/he become aware of any of the safety findings listed below. Occurrence of 1 or more of the following will result in immediate suspension of further IP administration (as applicable per the study timeline) at all study sites and review of the safety data by the SMC.

1. One or more subjects experience an SAE assessed as other than unrelated to IP by the investigator, sponsor, or SMC chair.
2. One or more subjects have an anaphylactic reaction within 4 hours of vaccination or that is assessed as other than unrelated to IP.
3. Three or more subjects have a Grade 3 AE (excluding local reactogenicity that decreases to below Grade 3 within 1 day, vital signs, and laboratory values) assessed as other than unrelated to IP.

4. Three or more subjects have clinically significant Grade 3 vital sign(s) assessed as other than unrelated that remain clinically significant and at Grade 3 or higher upon repeat testing (Refer to Section 11.1.2).
5. Three or more subjects have clinically significant Grade 3 laboratory value(s) assessed as other than unrelated that remain clinically significant and at Grade 3 or higher upon repeat testing (Refer to Section 11.1.8).
6. One or more subjects have a Grade 4 AE assessed as other than unrelated to IP.
7. A pattern of clinically significant symptoms, physical findings, AEs, or clinically significant laboratory abnormalities that in the opinion of the sponsor, medical monitor, or SMC chair, collectively represent a potential safety concern.

BARDA retains the right to suspend or end the study or to discontinue specific study groups at any time. In case of premature termination or suspension and safety review of the study, BARDA will inform regulatory authorities, and Rho will promptly inform the investigators of the termination or suspension of the study and the reason for termination or suspension.

7. SELECTION AND WITHDRAWAL OF SUBJECTS

7.1. Selection of Study Population

Subjects will be randomized to study treatment only if they meet all of the applicable inclusion criteria and none of the exclusion criteria. In addition, applicable eligibility criteria must be reviewed just prior to each vaccination; if the subject no longer meets applicable eligibility criteria, the investigator, in consultation with the medical monitor in cases of uncertainty, must determine whether the subject should receive the IP dose or be terminated early from study IP.

Deviations from the inclusion and exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability, or subject safety. Therefore, adherence to the criteria specified in the protocol is essential.

7.1.1. Subject Inclusion Criteria

1. Male or nonpregnant females, 66 years of age or older at the time of randomization for the elderly population, or 18 through 50 years of age at the time of randomization for the younger population.
2. Females who are of childbearing potential and are sexually active with a male partner must agree to use an acceptable method of birth control from Screening to Day 64 and must have used a reliable birth control method for at least 2 months prior to Screening.
    a. A female of childbearing potential is defined as post onset menarche and pre-menopausal female capable of becoming pregnant. This does not include females who meet any of the following conditions: menopausal >2 years, tubal ligation >1 year, bilateral salpingo-oophorectomy, or hysterectomy.
    b. Adequate contraception is defined as a contraceptive method with a failure rate of less than 1% per year when used consistently and correctly and when applicable, in accordance with the product label. Examples include: oral contraceptives, either combined or progestogen alone; injectable progestogen; implants of etenogestrel or levonorgestrel; estrogenic vaginal ring; percutaneous contraceptive patches; intrauterine device or intrauterine system; male partner sterilization at least 6 months prior to the female subject's Screening Visit, and this male is the sole partner for that subject (the information on the male partner's sterility can come from the site personnel's review of the subject's medical records or interview with the subject on her medical history); male condom combined with a vaginal spermicide (foam, gel, film, cream, or suppository); male condom combined with a female diaphragm, either with or without a vaginal spermicide (foam, gel, film, cream, or suppository).
3. Able to provide written informed consent prior to initiation of any study procedures. As part of the consent process, subjects must be able to demonstrate understanding by passing the "Assessment of Understanding Questionnaire" within 2 attempts. Passing is defined as being able to answer all questions correctly.
4. In relatively stable health based on site investigator's judgment, as determined by medical history, physical examination, and the following criteria:

a. Stable health for age (defined as no new conditions per medical history, new medications in a different therapeutic class, or change in daily dose of existing prescription medications within the 45 days preceding Screening). Effective treatment (to resolution) of an acute infection (e.g., urinary tract infection, cellulitis, otitis, or bronchitis) with an antibiotic within 45 days preceding Screening will not be considered a deviation from this inclusion criterion as long as the antibiotic therapy was completed at least one week prior to Screening and no signs or symptoms of the infection have been present since the completion of treatment. Any prescription change that is due to change of health care provider or insurance company, or that is made for reasons that do not reflect a change in disease status (e.g., financial considerations), as long as within the same general class of medication, will not be considered a deviation from this inclusion criterion. Any change in prescription medication due to improvement of a disease outcome, as determined by the site investigator, will not be considered a deviation from this inclusion criterion.

b. Subjects may be on chronic or as needed (prn) medications if, in the opinion of the site investigator, these pose no additional risk to subject safety or assessment of reactogenicity and immunogenicity and their use is not for management of a worsening of medical diagnosis or condition. Note: Topical, nasal, and inhaled medications (with the exception of steroids, as outlined in the subject exclusion criteria) and vitamins are permitted.

5. Have a body mass index (BMI) less than 35.0 kg/m^2 at Screening. (BMI will not be reassessed prior to subsequent vaccinations.)
6. Have access to a consistent and reliable means of telephone contact, which may be home, workplace, or by personal mobile electronic device.
7. Are available and can comply with all study visits.

7.1.2. Subject Exclusion Criteria

1. Females who have a positive urine pregnancy test at Screening or within 24 hours prior to each study vaccination or women who are breastfeeding.
2. Have a requirement for skilled nursing care.
3. Have a history of severe reactions to components of AVA or CPG 7909, e.g., formaldehyde, benzethonium chloride (phemerol), or aluminum.
4. Have a history of latex sensitivity.
5. Have a history of ever receiving a vaccine for anthrax prior to Screening, or had an anthrax infection.
6. Have any medical, psychiatric, or social condition that, in the opinion of the site investigator, unfavorably alters the risk-benefit of subject participation, or is likely to interfere with study compliance or assessments, or is likely to confound interpretation of safety or immunogenicity data.
7. Have any diagnosis, current or past, of a potentially immune-mediated medical condition, such as Guillain-Barré syndrome, narcolepsy, or an autoimmune or chronic inflammatory disease (Appendix 3).

8. Have any diagnosis, current or past, of schizophrenia or bipolar disorder.
9. Have been hospitalized for psychiatric illness, have a history of suicide attempt, or confinement for danger to self or others within the preceding 10 years prior to Screening and each study vaccination.
10. Have a history of alcohol or drug abuse (per investigator's judgment) within 5 years prior to Screening and each study vaccination, or test positive for drugs of abuse at Screening (including for cannabis, even where legal). Benzodiazepines for anxiety disorders and stimulants for attention deficit hyperactivity disorder are not exclusionary if the subject has been on a stable dose for more than 3 months prior to Screening and each study vaccination and if the subject can produce a valid, current prescription for the medication. Propoxyphene, opioids, or combinations containing these medications (including as used for opioid addiction) are not permitted regardless of prescription status. Note: A positive Screening urine drug screen may not be repeated. (Drug screen will not be repeated prior to subsequent vaccinations.)
11. Have known human immunodeficiency virus (HIV), or acute or chronic hepatitis B or hepatitis C infection based on medical history; or test positive for any of these at Screening. Subjects who have been effectively treated for hepatitis C, as evidenced by a negative hepatitis C ribonucleic acid (RNA) confirmation test and who no longer require antiviral therapy, are eligible for participation. (Screening tests will not be repeated prior to subsequent vaccinations.)
12. Have known active neoplastic disease or a history of any hematologic malignancy. However, subjects with superficial skin cancer who do not require intervention other than local excision are not excluded.
13. Have immunosuppression as a result of an underlying illness or treatment, or use of anticancer chemotherapy (cytotoxic) or radiation therapy within 3 years prior to Screening and each study vaccination.
14. Have taken any systemic immunosuppressive agents, including immunomodulators, 6 months prior to Screening and each study vaccination. Allergen immunotherapy (desensitization) is not exclusionary. Allergen immunotherapy (desensitization) does not include systemic antibodies such as benralizumab, mepolizumab, or omalizumab; these are exclusionary.
15. Are suffering from or have a history of neuralgia, paresthesia, or neuritis within 90 days prior to Screening and each study vaccination; or have any history of stroke.
16. Have had convulsions or encephalomyelitis within three years prior to Screening and each study vaccination.
17. Have received immunoglobulin or other blood products (with the exception of Rho[D] immune globulin) within 90 days prior to Screening and each study vaccination.
18. Have received an experimental agent within 180 days prior to the first study vaccination, or expects to participate in another clinical trial with an interventional agent during the study period. This includes licensed or unlicensed vaccines, drugs, biologics, devices, blood products, or medications. Participation in an observational study is not

exclusionary as long as it doesn't interfere with study visits or procedures (e.g., blood collection volume restrictions).

19. Have taken oral or parenteral corticosteroids of any dose within 30 days prior to each study vaccination.
20. Have taken high-dose inhaled corticosteroids within 30 days prior to each study vaccination. High-dose is defined as >800 mcg/day of beclomethasone dipropionate or equivalent. Lower doses of inhaled corticosteroids are not exclusionary.
21. Have received any licensed live vaccine within 30 days prior to the first study vaccination or planned receipt from the first study vaccination through Visit 10 (Day 64).
22. Have received any licensed inactivated vaccine within 14 days prior to the first study vaccination or planned receipt from the first study vaccination through Visit 10 (Day 64).
23. Have an acute illness, as determined by the site investigator, within 72 hours prior to each study vaccination.
    a. If subject's oral temperature is above 100.0°F (37.8°C), the subject may be re-assessed for eligibility within the visit window (+3 Days).
    b. An acute illness that is nearly resolved, with only minor residual symptoms remaining, is allowable if, in the opinion of the site investigator, the residual symptoms will not interfere with the ability of study staff to assess safety parameters as required by the protocol.
    b. For bacterial infections that have been successfully treated with antibiotics, inclusion criterion 4a applies.
24. Have a history of myocardial infarction or ischemia, or have electrocardiogram (ECG) findings of myocardial infarction, ischemia, strain, complete bundle branch block, or ventricular arrhythmias (other than unifocal premature ventricular contractions ≤2/minute). Additionally, other cardiac history or ECG findings, which in the opinion of the investigator represent a potentially unstable or unacceptably high risk of an adverse cardiac outcome, are exclusionary. (ECG will not be repeated prior to subsequent vaccinations.)
25. Have any laboratory test result or clinical findings (including vital signs) that singly or in combination are, in the investigator's opinion, likely to unfavorably alter the risk-benefit of subject participation or to confound study safety or immunogenicity results. Additionally, the following are exclusionary:
    a. Any clinically significant Grade 3 laboratory or vital sign result, or any Grade 4 laboratory or vital sign result (regardless of assessed significance) at Screening. For subsequent vaccinations, abnormal laboratory results and vital sign results post-vaccination will be assessed against the rules for early termination of dosing (Section 6.4.1).
    b. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >2 times the upper limit of normal (ULN), or bilirubin >1.5 times the ULN unless isolated Gilbert's syndrome.
    c. Creatinine >1.5 times ULN for age and sex.

d. White blood cell count <3,000/µL or >12,500/µL; absolute neutrophil count <1200/µL; absolute lymphocyte count <750/µL; hemoglobin <10 g/dL females, or <11.5 g/dL males; platelet count <75,000/µL.
e. Hemoglobin A1C (HbA1C) >7.0% at Screening. (HbA1C will not be repeated prior to subsequent vaccinations.)

Subjects cannot be rescreened for exclusionary laboratory test results. Potentially exclusionary vital sign results may be repeated, but unless they have resolved to a Grade 3 designation or less *and* are not considered clinically significant, the subject should be excluded. The most recent laboratory and vital sign results will be used when determining eligibility for subsequent vaccinations.

7.2. Subject Withdrawal Criteria

Every subject has the right to refuse participation in the study (i.e., withdraw consent) at any time without providing any reason for withdrawal. A subject's participation must be terminated immediately upon his/her request, and the reason(s) for discontinuation documented accordingly in the corresponding eCRF.

Any subject who meets 1 or more of the following criteria will be removed from the study without prejudice:

- Subject request.
- Subject noncompliance, defined as refusal or inability to adhere to the study protocol or any other instances determined by the investigator or BARDA.
- Subject lost to follow-up.
- Investigator no longer believes participation is in the best interest of the subject.
- At request of BARDA, regulatory agencies, or the institutional review board (IRB).

For any subject who meets the above criteria prior to completion of Visit 10 (Day 64), the early termination (ET) visit assessments should be performed as outlined in Appendix 1, when possible. If not possible to conduct a visit, site staff should make every effort to obtain updated safety information (AE assessment including pregnancy information) by phone.

7.2.1. Replacements

If a subject is withdrawn from the clinical study after receipt of the first dose of IP, the subject will not be replaced.

If a subject is randomized but does not receive the first dose of IP, the subject will be withdrawn and will not be counted toward the total enrollment goal. Additional subjects will be randomized to achieve enrollment goals.

8. TREATMENT OF SUBJECTS

8.1. Description of Study Investigational Products

8.1.1. BioThrax

BioThrax, manufactured by Emergent BioSolutions (Emergent) in the US, is a vaccine indicated for active immunization for the prevention of disease caused by *B. anthracis* in persons 18 to 65 years of age, inclusive. BioThrax is approved by the FDA for the following indications:

1. Pre-exposure prophylaxis of disease in persons at high risk of exposure to *B. anthracis*.
2. Post-exposure prophylaxis of disease following suspected or confirmed *B. anthracis* exposure, when administered in conjunction with recommended antibacterial drugs.

BioThrax is a sterile, milky-white suspension for IM or SC injection made from cell-free filtrates of microaerophilic cultures of an avirulent, non-encapsulated strain of *B. anthracis*. The production cultures are grown in a chemically defined protein-free medium consisting of a mixture of amino acids, vitamins, inorganic salts, and sugars. The final product, prepared from the sterile filtrate culture fluid contains proteins, including the 83kDa PA protein, released during the growth period and contains no dead or live bacteria. The final product is formulated to contain 1.2 mg/mL aluminum, added as aluminum hydroxide in 0.85% sodium chloride. The final product is formulated to contain 25 mcg/mL benzethonium chloride and 100 mcg/mL formaldehyde, added as preservatives.

BioThrax will be supplied in 5.0 mL multidose vials containing ten 0.5 mL doses. Each vial will only be used to administer a single dose.

8.1.2. AV7909 Anthrax Vaccine

AV7909 is being developed by Emergent as a next-generation anthrax vaccine indicated for PEP to reduce the risk of anthrax after exposure to *B. anthracis*.

AV7909 is a pre-formulated combination product containing AVA and CPG 7909 for IM injection. The AV7909 drug product is a sterile, milky-white suspension made from cell-free filtrates of microaerophilic cultures of an avirulent, non-encapsulated strain of *B. anthracis* that has been adsorbed to aluminum hydroxide + CPG 7909 adjuvant. The drug product is formulated to contain 1.2 mg/mL aluminum, added as aluminum hydroxide (Alhydrogel). It also contains 0.85% sodium chloride and, as preservatives, 25 mcg/mL benzethonium chloride and 100 mcg/mL formaldehyde.

The formulation contains 0.5 mL of AVA and 0.25 mg of CPG 7909 per dose. The amount of free (unbound) CPG 7909 per dose is expected to be ≤ 0.045 mg.

AV7909 is supplied in 6 mL (nominal fill volume) clear borosilicate glass multi-dose vials (approximately 6.1 mL/vial) with a rubber stopper and flip-top aluminum seal. Each vial will only be used to administer a single dose.

8.1.3. Placebo

Commercially available sterile, preservative-free sodium chloride for injection (Sodium Chloride Injection, USP 0.9%) will be used as placebo in the study. Each mL contains sodium chloride 9 mg and water for injection q.s. It contains no bacteriostat, antimicrobial agent, or added buffer. The solution may contain hydrochloric acid and/or sodium hydroxide for pH adjustment (pH 4.5-7.0).

Each 10.0 mL vial of sodium chloride will only be used to administer a single 0.5 mL dose. Additional information is provided in the Sodium Chloride Injection, USP package insert.

8.2. Concomitant Medications

Any treatment including all prescription drugs, herbal products, vitamins, minerals, and over-the-counter (OTC) medications or therapies administered from the time of consent through the end of study participation is considered a concomitant medication. Concomitant medication use will be recorded in the eCRF and will include the medication name, dose, frequency, route of administration, and the dates of administration. All concomitant medications used by the subject from the time of consent until Visit 9 (Day 50) will be recorded in the subject's eCRF. Following Visit 9 (Day 50), concomitant medications will only be recorded if they are used for the treatment of a MAAE, SAE, or PIMMC. Any changes, additions, and/or deletions in concomitant medications throughout the course of the subject's participation in the study will be recorded in the subject's eCRF.

If a subject is discovered to be using a prohibited concomitant medication after he or she is enrolled in the study, the investigator, in consultation with the medical monitor in cases of uncertainty, should determine the impact on the subject's participation. All instances of use of prohibited concomitant medications must be documented on the appropriate eCRFs.

In addition, any nonstudy vaccines received 30 days prior to the Screening Visit and nonstudy vaccines received through Visit 10 (Day 64) will be recorded in the subject's eCRF. Prior medications used within 30 days of the Screening Visit will also be recorded in the subject's eCRF.

8.2.1. Prohibited Concomitant Medications and Therapies

The following concomitant medications and therapies are prohibited for the duration of the study unless otherwise specified.

- Anticancer chemotherapy (cytotoxic) or radiation therapy.
- Systemic immunosuppressive agents, including immunomodulators. Allergen immunotherapy (desensitization) is not prohibited. Allergen immunotherapy (desensitization) does not include systemic antibodies such as benralizumab, mepolizumab, or omalizumab; these are prohibited.
- Oral or parenteral corticosteroids.
- High-dose inhaled corticosteroids (>800 mcg/day of beclomethasone di-iso-propionate CFC or equivalent).

- Treatment with immunoglobulin or other blood products (with the exception of Rho[D] immune globulin).
- Vaccines (including seasonal influenza vaccines) from screening through 35 days following the third dose of the study vaccine (Screening Visit through Visit 10 [Day 64]).
- Experimental agents (vaccine, drug, biologic, device, blood product, or medication).

8.2.2. Permitted Concomitant Medications

Other than the prohibited medications and therapies listed in Section 8.2.1, treatment with concomitant medications and therapies is permitted during the study. Concomitant medication deemed necessary for the welfare of the subject during the study may be given at the discretion of the investigator. However, it is the responsibility of the investigator to ensure that details regarding the medication will be recorded in full in the eCRF.

8.3. Treatment Compliance

The study IP will be administered by an unblinded study staff member and thus is an observed compliance. Subject compliance will be determined by the number and percentage of subjects who receive study IP on Day 1, Day 15, and Day 29 by study group. Any deviations from the dosing schedule outside the defined visit windows (Appendix 1) must be approved by the BARDA Medical Officer and will be recorded on the appropriate eCRF.

9. STUDY INVESTIGATIONAL PRODUCT MATERIALS AND MANAGEMENT

9.1. Study Investigational Product

Refer to Section 8.1 for detailed information regarding the BioThrax, AV7909, and placebo study IP.

9.2. Study Investigational Product Packaging and Labeling

Study IP will be packaged and labeled according to applicable local and regulatory requirements. Study IP will be provided in single-dose kits containing 1 vial of BioThrax, AV7909, or placebo. BioThrax, AV7909, and placebo will be provided in multi-dose vials; however, each vial will only be used to administer a single dose. Vials will be labeled with a single-panel, unblinded label. Each kit carton will be labeled with an unblinded label with a unique kit number (kit ID) and will contain 2 additional labels: 1 to be affixed to the site's study IP accountability documentation, and 1 to be affixed to the syringe used for IP administration.

The vials and kits will be labeled in English. The BioThrax and AV7909 vial labels and kit labels will include a note that specifies the following "Caution: New Drug Limited by Federal (or United States) Law to Investigational Use," as well as storage specifications. The placebo kit labels will include the Caution statement and storage specifications. In addition, the vial labels will contain the lot number.

9.3. Study Investigational Product Storage

Study IP must be stored in a secure area (e.g., a locked room or locked refrigerator), and protected from light and moisture. BioThrax and AV7909 should be kept at a controlled standard refrigeration temperature between 2° to 8°C (36° to 46°F), inclusive. Placebo should be kept between 20° to 25°C (68° to 77°F), inclusive. The temperature of the storage unit must be monitored and documentation of proper storage must be maintained. Investigational product should be removed from the storage unit and allowed to sit at room temperature for approximately 15 minutes before administration.

9.4. Study Investigational Product Preparation and Administration

Because BioThrax, AV7909, and placebo are distinguishable visually and by route of administration, unblinded study staff will be responsible for preparing and administering study IP. Subjects will be asked to look away while the vaccine is being administered.

Study IP should be visually inspected for particulate matter and discoloration during IP preparation and prior to administration; it should not be administered if either condition exists. Study IP vials should be shaken thoroughly to ensure that the suspension is homogeneous during withdrawal. BioThrax and AV7909 should have a milky-white appearance. Placebo should be clear. The treatment syringe barrel should be covered with the subject-specific label to obscure the contents.

BioThrax (0.5 mL) should be administered by SC injection over the deltoid muscle. AV7909 (0.5 mL) should be administered IM in the deltoid muscle. Placebo IP (0.5 mL) will be administered IM in the deltoid muscle. The 3 IP doses for a given subject should be administered in alternating arms. Investigational product doses can be administered in the same arm if a skin condition prevents administration in one of the arms (e.g., scar tissue, skin rash, etc.). The unblinded study staff will record which arm received each dose of IP.

9.5. Study Investigational Product Accountability

The investigator is required to maintain adequate records of the disposition of the study IP, including the date and quantity of IP received, to whom the IP was dispensed (subject-by-subject accounting), and a detailed accounting of any IP accidentally or deliberately destroyed. Records for receipt, storage, use, and disposition will be maintained by the study site. An IP dispensing log will be kept current and will include kit ID, identification of each subject, and the date and quantity of IP dispensed. All records regarding the disposition of the study product will be available for inspection by the unblinded study monitor.

9.6. Study Investigational Product Handling and Disposal

After dosing has been completed, to satisfy regulatory requirements regarding accountability, all study IP will be reconciled and returned to the study IP distributor according to applicable regulations. Study IP should not be destroyed until authorized in writing by BARDA.

9.7. Randomization and Blinding

9.7.1. Randomization

Subjects ≥66 years of age will be randomized 1:1:1:1 across 4 treatment arms, and subjects 18 through 50 years of age will be randomized 1:1 across 2 study arms as specified in Table 2. An interactive web response system (IWRS) will be used to centrally administer the randomization schedule. The randomization schedule will be generated using SAS software Version 9.3 or later (SAS Institute Inc., Cary, North Carolina). Randomization will take place according to a fixed schedule using a permuted block design stratified by sex and age group (18-50, 66-74, and ≥75 years). The IWRS will assign subjects to treatment arms based on the predefined randomization list. A kit ID corresponding to the assigned treatment will be assigned by the IWRS at randomization and at the Day 15 and Day 29 Visits from the inventory available at the site. The randomization schedule will be generated by the unblinded randomization team and will be kept strictly confidential, accessible only to authorized unblinded persons (Section 9.7.2).

9.7.2. Blinding

This is a double-blinded study. The study staff members who will prepare and/or administer the study IP will be unblinded to IP assignment. The unblinded study staff will not be involved with safety evaluations, nor will they perform subsequent assessments or engage in other study activities that could reveal the blind. Investigators, all investigational site staff (except those responsible for preparing/administering the IP), and all subjects participating in this study will be

blinded to IP assignment. Laboratory staff performing the safety laboratory assessments and immunogenicity assays will be blinded to study group.

To preserve the study blind, a minimum number of Rho and BARDA personnel will have access to treatment randomization information at the individual subject level. The unblinded Rho study team will include a statistician, statistical programmer, project manager or clinical team lead, study monitors, and the Client Support Services and IWRS teams. During monitoring visits, the unblinded study monitor(s) will perform study IP accountability and will check that the blind has been maintained. Blinded study monitor(s) will continuously assess the progress of the study but will not have access to the unblinded study records. A BARDA representative may be unblinded to assist with oversight of study IP management. In addition, as needed to meet regulatory reporting requirements, designated Rho and BARDA pharmacovigilance and/or regulatory personnel may be unblinded to the treatment status of individual subjects. In this circumstance, and if there are no other concerns, neither the BARDA project coordination team nor the study staff will be further unblinded to treatment status.

If an SMC meeting is needed (Section 6.4.2), the members of the SMC will review safety data fully unblinded at the study group and subject level during the closed session meeting. The BARDA and Rho blinded teams will review unblinded data at the study group level for the interim analysis (Section 12.1.4.4).

9.7.3. Breaking the Blind

Unblinding of individual subjects should occur only when knowledge of the treatment assignment will have a direct bearing on the medical treatment or evaluation of a subject. Whenever possible, the need to unblind should be discussed with BARDA and the medical monitor prior to unblinding. In the event of an emergency, the investigator or designated qualified individual may obtain the subject's blinded treatment via IWRS. If an individual designated to perform emergency unblinding does not have access to the IWRS, Rho Client Support Services may be contacted and can perform the unblinding for that individual. Upon performance of a blind break within the IWRS, the system will send out a blinded notification to alert Rho and BARDA staff of the event.

A full account of the unblinding event will be recorded in the subject's source document and eCRF, including the date and time of the unblinding, the reason for the decision to unblind, the extent of unblinding, and the name and signature of the individual who made the decision to unblind. The treatment assignment should not be included in either the source document or the eCRF.

Data for the interim analysis will be presented at the group level; thus, the blinding at the subject level will be maintained to those outside the unblinded team until database lock of all data through Visit 13 (Day 394) for the clinical study report. The blinded site and laboratory staff will be kept blinded at the subject level through the end of study.

10. ASSESSMENT OF IMMUNOGENICITY

Venous blood samples will be collected for immunogenicity assays at Visit 1 (Day 1) prior to vaccination, Visit 3 (Day 8), Visit 5 (Day 22), Visit 6 (Day 29) prior to vaccination, Visit 7 (Day 36), Visit 8 (Day 43), Visit 9 (Day 50), Visit 10 (Day 64), Visit 11 (Day 85), Visit 12 (Day 181), and Visit 13 (Day 394). Serum for immunogenicity assays will be collected at an ET or Unscheduled Visit only if within window for the next expected study visit at which serum is to be collected. Serum will be shipped to the central laboratory for storage and subsequently shipped to the immunogenicity laboratory for analysis. Good Laboratory Practice-compliant TNA and ELISA assays will be conducted to determine TNA and anti-PA IgG antibody titers, respectively.

The details for sample handling, processing, and shipping will be provided in the Laboratory Manual.

11. ASSESSMENT OF SAFETY

11.1. Safety Parameters

Safety will be evaluated utilizing the assessments defined in this section. If deemed clinically necessary, additional safety assessments not currently specified in the protocol may be performed at the discretion of the investigator in consultation with the medical monitor and BARDA.

11.1.1. Demographic/Medical History

In order to define a baseline for potential AEs, the demographics and medical history of each subject will be collected at the Screening Visit and recorded on the appropriate eCRFs.

11.1.2. Vital Signs

Vital sign measurements, including oral temperature, heart rate, respiratory rate, pulse oximeter reading and diastolic and systolic blood pressure (after the subject is seated for at least 5 minutes), will be collected at the Screening Visit, Visit 1 (Day 1) pre-vaccination and post-vaccination, Visit 2 (Day 3), Visit 3 (Day 8), Visit 4 (Day 15) pre-vaccination and post-vaccination, Visit 5 (Day 22), Visit 6 (Day 29) pre-vaccination and post-vaccination, Visit 7 (Day 36), Visit 9 (Day 50), Visit 10 (Day 64), ET Visit, and Unscheduled Visit and will be recorded on the appropriate eCRF. Vital signs should be taken prior to any blood draws. Post-vaccination vital signs will be taken 30 minutes (±5 minutes) and 1 hour (±5 minutes) post-vaccination prior to discharge. Respiratory rate, if regular, may be assessed over 30 seconds and doubled, but in no case should it be assessed over a period of less than 30 seconds. Heart rate, if performed manually, must be assessed over at least 15 seconds.

Post-vaccination clinically significant Grade 3 and Grade 4 abnormal vitals should be reassessed prior to subsequent vaccinations, including the subject attending an unscheduled visit as needed.

As the subject will self-assess oral temperature between clinic visits when solicited AEs are being collected (within 8 days after each vaccination, inclusive of the vaccination day) (see Section 11.2.1.2), sites will train each subject at Visit 1 (subjects will take their own temperature in front of site staff to confirm correct measurement before leaving the clinic). Diary cards (Section 11.1.10) will include instructions, and sites will reinforce proper assessment at each applicable visit to continually assess subject competency.

11.1.3. Height and Weight

Subject's height and weight will be taken and BMI calculated at the Screening Visit and recorded on the appropriate eCRF.

11.1.4. Physical Examination

A physical examination will be performed at the Screening Visit to assess and confirm eligibility. The examination will include a general assessment of the skin, head, ears, eyes, nose, throat, neck, thyroid, lungs, heart, abdomen, lymph nodes, and musculoskeletal

system/extremities, and a neurological examination; the results will be recorded on the appropriate eCRF.

A symptom-directed physical examination will be performed at Visit 1 (Day 1), Visit 2 (Day 3), Visit 3 (Day 8), Visit 4 (Day 15), Visit 5 (Day 22), Visit 6 (Day 29), Visit 7 (Day 36), Visit 9 (Day 50), Visit 10 (Day 64), ET Visit, and Unscheduled Visit. Results will be recorded on the appropriate eCRF.

11.1.5. Electrocardiogram

A standard 12-lead ECG will be recorded and assessed at the Screening Visit, Visit 7 (Day 36), and Visit 13 (Day 394). The ECG will be reviewed by a medically qualified individual to confirm review of the ECG and verify whether any abnormalities are clinically significant. In general, abnormal, clinically significant ECGs are expected to be associated with an item recorded in the subject's medical history or with an AE.

11.1.6. High-sensitivity C-Reactive Protein

Serum samples will be collected for high-sensitivity C-reactive protein (hs-CRP) assay only in subjects ≥66 years of age at Visit 1 (Day 1) prior to vaccination and at Visit 2 (Day 3). The samples will be shipped to the central laboratory for analysis. The details for sample handling, processing, and shipping will be provided in the Laboratory Manual.

11.1.7. Autoantibody Sample Collection

Serum samples will be collected at Visit 1 (Day 1) prior to vaccination, and at Visit 10 (Day 64) or at an ET Visit prior to Day 64. The samples will be shipped to the central laboratory and will be frozen for potential testing of antinuclear antibodies, anti-double-stranded deoxyribonucleic acid (anti-dsDNA), and rheumatoid factor titers if needed. The details for sample handling, processing, and shipping will be provided in the Laboratory Manual.

11.1.8. Clinical Laboratory Assessments

Venous blood and urine samples will be collected for routine clinical laboratory safety evaluations at the Screening Visit, Visit 3 (Day 8), Visit 5 (Day 22), Visit 7 (Day 36), and at ET and Unscheduled Visits (if deemed clinically indicated by the investigator), and will be shipped to the central laboratory for analysis. Post-vaccination Grade 3 and Grade 4 abnormal laboratory results will be evaluated with an unscheduled repeat test. Repeat tests should be scheduled to ensure test results can be assessed in time to avoid an impact on the protocol-defined window for administration of Dose 2 or Dose 3. The details for sample handling, processing, and shipping will be provided in the Laboratory Manual.

Individual results will be sent to the applicable site, and the investigator will perform a clinical assessment of all laboratory safety data to assess eligibility and identify and document AEs as applicable. All results will be transferred electronically directly from the central laboratory to Rho using standard secure data transfer procedures.

The clinical laboratory assessments planned for this study include chemistry, hematology, and urinalysis assessments as follows.

11.1.8.1. Chemistry

- ALT
- AST
- Blood urea nitrogen
- Creatinine
- Glucose, random, serum
- Total bilirubin (fractionated for values for total bilirubin > ULN)

11.1.8.2. Hematology

Table 3 lists the parameters of the complete blood count with differential.

Table 3: Complete Blood Count with Differential

Red blood cells	**White blood cells**
Hematocrit	Basophils (% and absolute count)
Hemoglobin	Eosinophils (% and absolute count)
Red blood cell count	Lymphocytes (% and absolute count)
Platelets	Monocytes (% and absolute count)
Platelet count	Neutrophils (% and absolute count)
	White blood cell count

11.1.8.3. Urine Drug Screen and Urinalysis

Urine will be collected for a drug screen at the Screening Visit. The urine drug screen includes tests for amphetamines, barbiturates, benzodiazepines, cocaine, tetrahydrocannabinol, methylenedioxymeth-amphetamine, opiates, oxycodone, phencyclidine, and propoxyphene.

Urine will be collected at the Screening Visit, Visit 3 (Day 8), Visit 5 (Day 22), Visit 7 (Day 36), and at ET and Unscheduled Visits (if deemed clinically indicated by the investigator) for urinalysis. Urinalysis parameters include appearance, color, pH, specific gravity, ketones, protein, glucose, bilirubin, nitrite, urobilinogen, and occult blood.

11.1.9. Pregnancy Screen

At the Screening Visit, Visit 1 (Day 1), Visit 4 (Day 15), and Visit 6 (Day 29), a urine dipstick pregnancy test will be performed onsite for female subjects of childbearing potential. For Visits 1, 4, and 6, the test must be performed within 24 hours prior to vaccination, and results must be final and negative prior to vaccination.

Additionally, a urine dipstick pregnancy test should be performed at any time during study participation if pregnancy is suspected.

11.1.10. Diary Cards

All subjects will be provided with diary cards (see Table 4 and Figure 2) to aid in their recording of solicited local and systemic symptoms to the study IP and other unsolicited symptoms, including start and stop dates (see Section 11.1.12 and Section 11.2.1.2). In addition, the diary

cards will include training information on temperature measurements (Section 11.1.2) and injection site reaction assessment.

The investigator or his/her designee will review the information from the diary card or from memory with the subject at each applicable visit and assess whether any AE criteria are met based on FDA's standard toxicity grading scales (Section 11.3.3 and Appendix 2). Once confirmed to be an AE, the investigator or designee will enter the information into the appropriate eCRF. In addition, any changes in concomitant medications will be documented on the appropriate eCRF.

The diary cards will be collected from the subjects and stored as source documents.

Table 4: Schedule of Diary Cards

Diary Card	Type of Event	Vaccination	Collection Period: Days After Dosing	Collection Period: Study Days
Diary Card 1A	Solicited and Unsolicited	Dose 1	Days 1-8 Post-vaccination, inclusive of vaccination day	Days 1-8
Diary Card 1B	Unsolicited	Dose 1	Days 8-15 Post-vaccination	Days 8-15
Diary Card 2A	Solicited and Unsolicited	Dose 2	Days 1-8 Post-vaccination, inclusive of vaccination day	Days 15-22
Diary Card 2B	Unsolicited	Dose 2	Days 8-15 Post-vaccination	Days 22-29
Diary Card 3A	Solicited and Unsolicited	Dose 3	Days 1-8 Post-vaccination, inclusive of vaccination day	Days 29-36
Diary Card 3B	Unsolicited	Dose 3	Days 8-21 Post-vaccination	Days 36-50

11.1.11. Post-vaccination Evaluation

At Visit 1 (Day 1), Visit 4 (Day 15), and Visit 6 (Day 29), subjects will be monitored for at least 1 hour after vaccination; any safety events and concomitant medications will be recorded on the appropriate eCRFs. Details regarding collection and reporting of solicited local and systemic reactogenicity symptoms are presented in Section 11.1.10, Section 11.1.12 and Section 11.2.1.2.

The following evaluations will be performed:

1. Vital sign measurements will be obtained at 30 minutes (±5 minutes) and 1 hour (±5 minutes) post-vaccination as described in Section 11.1.2.
2. Injection site evaluation will be completed at 1 hour (±5 minutes) post-vaccination (Section 11.1.12).

11.1.12. Injection Site Evaluation

Injection site evaluations will be performed to assess injection site warmth, tenderness, itching, pain, restriction of range of arm motion, erythema/redness, palpable or observable lump, induration/swelling, and bruising. The Dose 1 injection site will be examined at Visit 1 (Day 1) at 1 hour (±5 minutes) post-vaccination, Visit 2 (Day 3), Visit 3 (Day 8), and at Visit 4 (Day 15 prior to Dose 2 vaccination). The Dose 2 injection site and the Dose 1 injection site (contralateral arm, if applicable) will be examined at Visit 4 (Day 15) at 1 hour (±5 minutes) post-vaccination, Visit 5 (Day 22), and Visit 6 (Day 29 prior to Dose 3 vaccination). The Dose 3 injection site and the Dose 2 injection site (contralateral arm, if applicable) will be examined at Visit 6 (Day 29) at 1 hour (±5 minutes) post-vaccination, Visit 7 (Day 36), and Visit 8 (Day 43). Results will be recorded on the appropriate eCRF.

If a visible injection site abnormality is observed,

1. The abnormality will be documented with a photograph if the subject consented.
2. The area (largest diameter) of erythema/redness and/or induration/swelling will be measured and documented.

Photographs will be taken by study staff only in the event of abnormal findings for accurate description and evaluation of the findings and safety reviews, and will be retained with subject source documents. There are no digital format requirements; the photographs will not be stored in the study database and will not be used as part of the safety analysis. They may, however, be shared with the medical monitor, BARDA, and the SMC in a secure and blinded manner without exposing personally identifiable information.

11.1.13. Adverse Event Assessment

Site staff will ask nonleading questions regarding the subject's health status (Section 11.3.1) and document any new or changed AEs on the appropriate eCRF. Due to the known increased risk of the elderly population to cardiac and neurovascular events, the need for special attention to these AEs in this population will be stressed at investigator meetings and site trainings.

11.1.14. Concomitant Medication Assessment

Site staff will review concomitant medications (Section 8.2) and document any new or changed medications on the appropriate eCRF.

11.2. Adverse and Serious Adverse Events

11.2.1. Definition of Adverse Events

11.2.1.1. Adverse Events

An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not it is considered drug related [21 Code of Federal Regulations [CFR] 312.32(a)]. 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, whether or not related to the medicinal (investigational) product (Integrated Addendum

to International Conference on Harmonisation (ICH) E6[R1]: Guideline for Good Clinical Practice (GCP) E6[R2]).

Laboratory results and vital sign excursions of any magnitude will be defined as AEs if they are considered clinically significant by the investigator. Any Grade 4 laboratory value should be considered an AE.

Suspected adverse reaction (SAR) means any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. A SAR implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by a drug [21 CFR 312.32(a)].

An AE or SAR is considered "unexpected" if it is not listed in the BioThrax package insert or AV7909 investigator brochure or is not listed at the specificity or severity that has been observed.

11.2.1.2. Solicited and Unsolicited Adverse Events

For the purposes of this study, local reactions and specific systemic AEs will be solicited from the subject for 8 days post-vaccination, inclusive of the vaccination day (Section 11.1.10).

- Solicited local injection site and prior injection site reactions are defined in Section 11.1.12.
- Solicited systemic reactions will include fatigue, myalgia/muscle ache, headache, and fever.

All other AEs reported by the subject will be defined as unsolicited AEs. All unsolicited AEs during the early part of the study (through Day 50, Section 11.1.10) will be recorded. Following Day 50, unsolicited AEs will only be recorded if they are defined as SAEs, MAAEs, or PIMMCs.

11.2.1.3. Serious Adverse Events

An AE or SAR is considered serious if, in the view of either the investigator or BARDA, it results in any of the following outcomes [21 CFR 312.32(a)]:

- Death.
- Life-threatening AE that, in the view of the investigator or BARDA, places the subject at immediate risk of death. This does not, however, include an event that might have caused death had it occurred in a more severe form.
- Requires inpatient hospitalization or prolongs existing hospitalization. Planned hospitalizations will **not** be reported as SAEs unless categorized as medically important. Emergency room visits and observational admissions of under 24 hours, in themselves, do not qualify as SAEs.
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions.
- Congenital anomaly/birth defect.

- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience, when based on appropriate medical judgment, they may jeopardize the subject or the subject may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

11.2.1.4. Medically Attended Adverse Events

The FDA requires that clinical trials of preventive vaccines with CPG 7909 adjuvant implement collection and analysis of data relating to MAAEs among subjects in all study groups from the time of the first dose of study IP through 12 months following the last dose of study IP, due to the theoretical potential for induction of autoimmune or autoinflammatory diseases.

MAAEs are defined as AEs with medically attended visits including hospital, emergency room, urgent care clinic, or other visits to or from medical personnel for any reason. Adverse events (e.g., abnormal vitals) identified at a routine study visit will not be considered MAAEs.

11.2.1.5. Potentially Immune-Mediated Medical Conditions

The FDA requires that clinical trials of preventive vaccines with CPG 7909 adjuvant implement collection and analysis of data relating to PIMMCs among subjects in all study groups from the time of the first dose of study IP through 12 months following the last dose of study IP, due to the theoretical potential for induction of autoimmune or autoinflammatory diseases.

For this study, the occurrence of a PIMMC is considered to be unexpected and will be reported as a serious and unexpected suspected adverse reaction (SUSAR) per 21 CFR 312.32.

A list of PIMMCs defined for this study is presented in Appendix 3.

11.3. Collection, Recording, and Grading Severity of Adverse Events

11.3.1. Collection of Adverse Events

AEs of any type described above may be discovered through a variety of methods:

- Observing the subject
- Reviewing and discussing the diary cards with the subject (Section 11.1.10)
- Questioning the subject with standard nonleading questions to elicit any medically related changes in their well-being (e.g., Have you been hospitalized, had any medical problems, used any new medications, or changed or stopped any medications [both prescription and OTC]?)
- Receiving an unsolicited complaint from the subject
- An abnormal value or result from a clinical (e.g., vital signs) or laboratory evaluation

11.3.2. Recording of Adverse Events

Throughout the study, the investigator will record AEs on the appropriate eCRF. Any clinically significant safety assessments that are associated with an underlying disease present at screening, unless judged by the investigator to be more severe than expected for the subject's condition, are

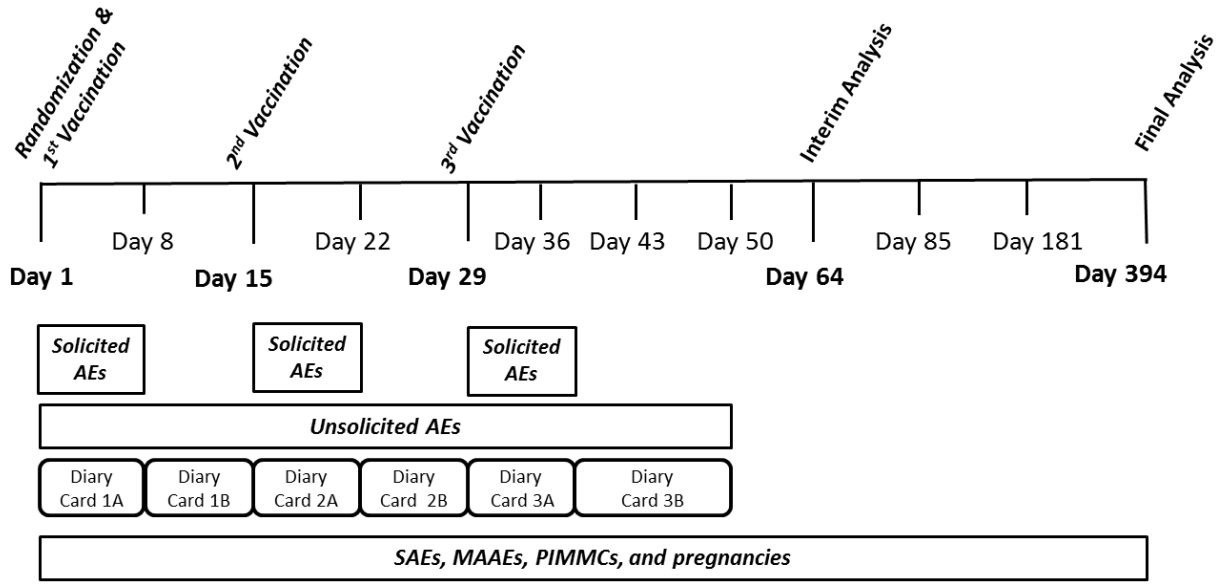
not to be reported as AEs or SAEs. Due to expectedness of mild fever, and mild redness and swelling at the injection site, any symptoms of redness, swelling and fever that do not meet the Grade 1 lower thresholds in Table 8 are **not** to be reported as AEs.

Treatment-emergent AEs (TEAEs) will be recorded during the periods defined below.

- Solicited AEs:
    - Those occurring within 8 days after each vaccination, inclusive of the vaccination day (Day 1 through Day 8, Day 15 through Day 22, and Day 29 through Day 36).
    - Those judged related to study IP will be followed to resolution (with or without sequelae) or until considered stable by the investigator (in consultation with the medical monitor in situations of uncertainty).
    - Those judged unrelated to study IP will be followed to resolution (with or without sequelae) or, if not resolving, until considered stable by the investigator (in consultation with the medical monitor in situations of uncertainty), or until the end of the subject's study participation, whichever comes first.
- Treatment-Emergent Unsolicited AEs:
    - All AEs other than solicited local and systemic reactogenicity symptoms occurring from the time the subject receives the first vaccination until Visit 9 (Day 50). Following Day 50, unsolicited AEs will only be recorded if they are defined as SAEs, MAAEs, or PIMMCs.
    - Those judged related to study IP will be followed to resolution (with or without sequelae) or until considered stable by the investigator (in consultation with the medical monitor in situations of uncertainty).
    - Those judged unrelated to study IP will be followed to resolution (with or without sequelae) or, if not resolving, until considered stable by the investigator (in consultation with the medical monitor in situations of uncertainty), or until the end of the subject's study participation, whichever comes first.
- Treatment-Emergent SAEs, MAAEs, and PIMMCs:
    - Those occurring from the time the subject receives the first vaccination until exit from the study.
    - SAEs and PIMMCs will be reported to Rho pharmacovigilance personnel within 1 business day of the site's awareness of the event. Only MAAEs meeting SAE criteria must be reported within 1 business day.
    - Those judged related to study IP will be followed to resolution (with or without sequelae) or until considered stable by the investigator (in consultation with the medical monitor in situations of uncertainty).
    - Those judged unrelated to study IP will be followed to resolution (with or without sequelae) or, if not resolving, until considered stable by the investigator (in consultation with the medical monitor in situations of uncertainty), or until the end of the subject's study participation, whichever comes first.

Figure 2 provides the safety data reporting periods used in this study.

Figure 2: Safety Data Reporting Periods



AE= adverse event; MAAE = medically attended adverse event; PIMMC = potentially immune-mediated medical condition; SAE = serious adverse event

Note: All unsolicited AEs will be collected through the end of the Visit 9 study visit window (i.e., Day 50, 21 days after the third vaccination). Following Day 50, unsolicited AEs will only be recorded if they are defined as SAEs, MAAEs, or PIMMCs.

Note: The entire study period is from Day 1 from the time a subject receives the first study vaccination through Visit 13 (Day 394).

Note: Pregnancies that occur during a subject's participation in the study will be followed until conclusion even if the pregnancy ends after Visit 13 (Day 394).

11.3.3. Grading Severity of Adverse Events

The severity of AEs and SAEs will be graded as defined by the FDA's standard toxicity grading scales[14] (Appendix 2). For the local and systemic solicited AEs that are not included in Appendix 2 (i.e., injection site warmth, restriction of range of arm motion, palpable or observable lump, and bruising), the severity grading will be based upon the subject's individual assessment of the extent to which the AE interfered with his/her regular daily activities as follows: Grade 0 (Absent)= symptom not present; Grade 1 (Mild) = symptom present but does not interfere with activities of daily living; Grade 2 (Moderate) = symptom causes some interference with activities of daily living: Grade 3 (Severe) = symptom prevents activities of daily living or requires treatment.

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity, whereas seriousness is defined by the criteria in Section 11.2.1.3.

11.4. Relationship and Attribution to Study Investigational Product

An investigator's causality assessment is the determination of whether a reasonable possibility exists that study IP caused or contributed to an AE, and must be provided for all AEs (serious

and non-serious). The investigator will assess the causality/relationship between the study IP and the AE and record that assessment in the appropriate eCRF.

BARDA's determination of attribution will be used for reporting to the appropriate health and regulatory authorities.

The relation and attribution of an AE to study treatment will be determined using the descriptors and definitions provided in Table 5.

Table 5: Attribution of Adverse Events

Unrelated Categories	
Not Related	The AE is clearly not related to study IP.
Unlikely Related	The AE is unlikely related to study IP.
Related Categories	
Possibly Related	The AE has a reasonable possibility to be related to study IP; evidence exists to suggest a causal relationship.
Probably Related	The AE is likely related to study IP.
Related	The AE is clearly related to study IP.

AE = adverse event; IP = Investigational Product

11.5. Reporting Safety Events to Regulatory Authorities

Once AEs, SAEs, and PIMMCs are recorded into the appropriate eCRF, Rho pharmacovigilance personnel will collaborate with BARDA and appropriate personnel to process and report events to the appropriate regulatory authorities within applicable regulatory timeframes. Procedures for AE processing and reporting are detailed in the Data Safety Monitoring Plan.

The sponsor will report to the FDA any suspected adverse reaction that is both serious and unexpected (SUSAR). The sponsor will report an AE as a SAR only if evidence exists to suggest a causal relationship between the study IP and the AE, such as:

a) A single occurrence of an event that is uncommon and known to be strongly associated with IP exposure (e.g., angioedema, Stevens-Johnson syndrome, Guillain-Barré syndrome).

b) One or more occurrences of an event that is not commonly associated with IP exposure, but is otherwise uncommon in the population exposed to the IP (e.g., narcolepsy).

c) An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of treatment) that indicates those events occur more frequently in the study group than in a concurrent or historical control group [21CFR 312.32(a)].

d) PIMMCs (Section 11.2.1.5) will be submitted as SUSARs.

11.6. Pregnancy Reporting

This study includes pregnancy as safety data. Although pregnancy is not an SAE, information about any pregnancy in a female study subject should be reported promptly to Rho on the same timeline as an SAE for tracking purposes (Section 11.3.2).

Study vaccination will be discontinued for the pregnant subject. The investigator shall counsel the pregnant subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the pregnant subject shall continue until the conclusion of the pregnancy.

The pregnancy will be documented on the appropriate eCRF when identified and at its conclusion.

Should pregnancy result in a congenital abnormality, birth defect, miscarriage, or medically indicated abortion, an SAE must be submitted to Rho using the SAE reporting procedures described in Section 11.3.2.

11.7. Reporting Other Safety Information

Investigators should promptly notify Rho, BARDA, and the IRB when an unanticipated problem involving risks to subjects or others is identified, which is not otherwise reportable as an AE.

12. STATISTICS

12.1. Endpoints

12.1.1. Primary Endpoints

There are 2 primary endpoints for this study.

- All solicited local and systemic reactogenicity symptoms occurring within 8 days after each vaccination, inclusive of the vaccination day, excluding reactogenicity symptoms on the contralateral arm.
- Seroprotection at Day 64, defined as a TNA NF_{50} antibody level ≥0.56.

12.1.2. Secondary Endpoints

12.1.2.1. Secondary Safety Endpoints

- All treatment-emergent unsolicited AEs.
- All treatment-emergent SAEs occurring during study participation.
- All treatment-emergent MAAEs occurring during study participation.
- All treatment-emergent PIMMCs occurring during study participation.
- All solicited local reactogenicity symptoms on the contralateral arm occurring within 8 days after each vaccination, inclusive of the vaccination day.

12.1.2.2. Secondary Immunogenicity Endpoints

- TNA NF_{50} antibody levels at Days 1, 8, 22, 29, 36, 43, 50, 64, 85, 181, and 394.
- TNA ED_{50} antibody levels at Days 1, 8, 22, 29, 36, 43, 50, 64, 85, 181, and 394.
- ELISA anti-PA IgG antibody levels at Days 1, 8, 22, 29, 36, 43, 50, 64, 85, 181, and 394.
- Seroprotection based on TNA NF_{50} at Days 1, 8, 22, 29, 36, 43, 50, 85, 181, and 394.
- Seroconversion based on TNA NF_{50} at Days 8, 22, 29, 36, 43, 50, 64, 85, 181, and 394, defined as a ≥4-fold increase over baseline levels, or a ≥4-fold increase over the LLOQ if the baseline value is below the LLOQ.
- Seroconversion based on TNA ED_{50} at Days 8, 22, 29, 36, 43, 50, 64, 85, 181, and 394.
- Seroconversion based on ELISA anti-PA IgG at Days 8, 22, 29, 36, 43, 50, 64, 85, 181, and 394.

12.1.3. Measures to Minimize Bias

Stratified block randomization will be performed centrally and will balance enrollment between treatment arms within the same age group and within each sex (see Section 9.7.1 for more

details). Clinical staff observing the subject post-vaccination, laboratory personnel analyzing samples, and study subjects will be blinded to study group (Section 9.7.2). Site staff will be trained in procedures to ensure blinding is maintained for all blinded individuals.

An independent unblinded team (Section 9.7.2) will generate, interpret, and report each analysis using appropriate study groups and will not modify any programs used to perform analyses.

12.1.4. Analysis Plan

Statistical analyses will be performed using SAS® software Version 9.3 or later.

Due to the exploratory nature of the primary objectives, inferential analyses are planned only as part of the secondary analyses.

Descriptive statistics (such as medians, quartiles, and ranges for continuous data, and percentages for categorical data) will be used to summarize subject characteristics, safety, and immunogenicity parameters. These summaries will be presented overall and separately for the subjects in the different study groups, as well as for pooled groups by treatment arm (regardless of age group) and by age group (regardless of treatment arm).

Details of the statistical analyses, methods, and data conventions will be described in the Statistical Analysis Plan (SAP).

12.1.4.1. Analysis Populations

12.1.4.1.1. Safety Population

The safety population will include all subjects who are randomized and receive at least 1 IP dose. Each subject will be analyzed as part of the study group corresponding to the actual treatment received for the applicable dose for individual dose summaries. For all other analyses by study group, each subject will be analyzed corresponding to the actual treatment received for the first dose. The safety population will be used for all safety analyses.

12.1.4.1.2. Immunogenicity Full Analysis Population

The immunogenicity full analysis population (IFAP) will include all subjects who are randomized, receive at least 1 IP dose, and have at least one determinate assay result at any post-vaccination visit. Each subject will be analyzed based on the treatment and dosing schedule actually received, regardless of the treatment arm assignment. The IFAP will be used only for analysis of seroprotection based on TNA NF_{50} antibody levels at Day 64 as a secondary analysis. Any additional immunogenicity analyses on the IFAP will be defined in the SAP.

12.1.4.1.3. Immunogenicity Per Protocol Population

The immunogenicity per protocol population (IPPP) will include all subjects in the IFAP who meet the following criteria:

- Receive a full dose of IP at Day 1, Day 15, and Day 29 within protocol specified visit windows (- 1 Day, +3 Days).
- Receive the correct treatment as assigned by randomization at Day 1, Day 15, and Day 29.

- Have no major protocol deviations that may have an impact on immunogenicity assessments.
- Have determinate assay results at the Day 64 visit.

The IPPP will be used for all primary and secondary immunogenicity endpoint assessments.

12.1.4.2. Primary Analyses

12.1.4.2.1. Primary Safety Analyses

The frequency of solicited local and systemic reactogenicity symptoms, as defined in Section 11.1.12 and Section 11.2.1.2, will be summarized by system organ class and preferred term. The risk of the event in each study group will be described using event rates and corresponding 95% CIs. Events will be included in the primary endpoint only if they occur within 8 days of vaccination, inclusive of the vaccination day (i.e., Day 1 through Day 8, Day 15 through Day 22, and Day 29 through Day 36), excluding reactogenicity symptoms on the contralateral arm.

12.1.4.2.2. Primary Immunogenicity Analyses

Proportions of subjects who achieve seroprotection based on TNA NF_{50} antibody levels at Day 64 will be reported for each study group with the corresponding 95% CI for that group.

12.1.4.3. Secondary Analyses

The 5 secondary safety endpoints (Section 12.1.2.1) will be summarized in the same manner as the primary safety endpoint. Additionally, both primary and secondary safety endpoints will be summarized by vaccination dose (i.e., Dose 1, 2, or 3), onset days post each vaccination, grade, duration, and relationship to study IP.

Continuous TNA NF_{50}, TNA ED_{50}, and ELISA anti-PA IgG antibody levels will be summarized as geometric mean levels by study group and visit with the corresponding 95% CIs about the geometric mean for each group.

Secondary immunogenicity endpoints related to seroprotection and seroconversion will be summarized in the same manner as the primary immunogenicity endpoint. Definitions of seroprotection and seroconversion can be found in Section 12.1.1 and Section □.

Comparisons between study groups in binary outcomes will be made using either a Pearson chi-square test or a Fisher's exact test, as appropriate. Rate differences will be reported, along with 95% CIs and p-values. Continuous outcome comparisons between study groups will be made using a *t*-test, and geometric mean ratios with corresponding 95% CIs and p-values will be reported.

Additional information regarding exploratory analyses, listings, and tabular summaries of safety assessments and immunogenicity measures will be specified in the SAP.

12.1.4.4. Interim Analysis

An interim analysis will be performed based on cumulative immunogenicity and safety data through Day 64 for all subjects. At the interim analysis, the study database (all data through Day 64) will be monitored and cleaned per the Data Management Plan (DMP). Data for the

interim analysis will be presented solely at the group level, thus, the blinding at the subject level will be maintained for those outside of the unblinded team until database lock of all data through Day 394 for the clinical study report.

All primary and secondary endpoint analyses up through Day 64 will be performed for the interim analysis as specified in Section 12.1.4.2 and Section 12.1.4.3. Since all subjects will have completed Day 64 and no formal statistical comparisons are being made as part of the primary objectives, there will be no penalty for an early look at the data.

Further details will be specified in the SAP.

12.1.4.5. SMC Reviews

The SMC will perform a planned review of interim safety data after 40 subjects ≥66 years of age have completed Visit 7 (Day 36). Enrollment and dosing will continue during this planned SMC review. In addition, ad hoc reviews will occur in the event that pausing/stopping rules are met or a review is deemed necessary by the SMC chair (Section 6.4.2).

The final SMC safety displays will be provided to the SMC members when the last study subject completes Visit 10 (Day 64).

Data displays for each SMC review will be generated by an unblinded statistician. Safety analyses for this study will be descriptive rather than inferential. Detailed listings and summary tabulations will be generated as specified in the SMC charter. The safety analyses will be completed using the safety population.

12.1.4.6. Final Analyses

A clinical study report will be written to include all safety and immunogenicity data through Day 394. For the final analysis, the study database will be monitored, cleaned, and locked per the DMP. Study data will be unblinded to prepare the study report. Further details will be specified in the SAP.

12.1.4.7. Exploratory Analyses

Exploratory analyses will be defined in the SAP, if applicable.

12.2. Sample Size Considerations

The sample size for this study is approximately 300 subjects: 200 subjects ≥66 years of age (older cohort) and 100 subjects 18 through 50 years of age (younger cohort). Approximately 50 subjects will be randomized into each of 6 study groups.

The proposed sample size for this study is based on previous experience with similar BioThrax studies in individuals aged 18 to 65 years. The previous studies had study arms of similar size that proved to be sufficient to allow the collection of meaningful data, especially with respect to local and systemic reactogenicity symptoms and antibody levels.

Though no hypothesis testing will be performed as part of the primary analyses, Table 6 and Table 7 show the probability of observing a safety event and statistical power to detect crossing a threshold in seroprotection rate, respectively, under a variety of hypothetical scenarios. Within each table, separate calculations are performed for each pooled group of interest.

Probabilities of detecting a safety event were calculated using the binomial distribution (Table 6).

Table 6: Probability of Observing at Least 1 Safety Event of Interest under Different True Event Rates

True Probability of a Safety Event of Interest	Per Study Group (N=50)	Pooled Study Groups (N=100)	Pooled ≥66 Years of Age Cohort (N=200)	Overall (N=300)
0.0001	0.005	0.010	0.020	0.030
0.005	0.222	0.394	0.633	0.778
0.01	0.395	0.634	0.866	0.951
0.02	0.636	0.867	0.982	0.998
0.05	0.923	0.994	>0.999	>0.999
0.1	0.995	>0.999	>0.999	>0.999

A lower bound of 40% is considered to be a meaningful threshold of success for the seroprotection rate for BioThrax.[4] Assuming a one-sided alpha level of 0.025, an exact binomial test was used to calculate the power to detect the lower bound of an exact 95% CI about an observed seroprotection rate being ≥40%, using sample size and hypothetical true seroprotection rate scenarios shown in Table 7.

Table 7: Power for Detecting a Lower 95% Confidence Limit of ≥40% about an Observed Seroprotection Rate

Hypothetical True Seroprotection Rate (%)	Per Study Group (N=50)	Pooled Study Groups (N=100)	Pooled ≥66 Years of Age Cohort (N=200)	Overall (N=300)
50	0.240	0.460	0.782	0.926
60	0.766	0.973	>0.999	>0.999
70	0.988	>0.999	>0.999	>0.999
80	>0.999	>0.999	>0.999	>0.999

12.3. Statistical Considerations

12.3.1. Covariates

Since no statistical models will be used for the primary and secondary endpoints, adjustments for covariates will not be necessary.

12.3.2. Multi-center Studies

By-site analyses planned for this study will be defined in the SAP.

12.3.3. Multiple Comparisons and Multiplicity

All safety and immunogenicity comparisons of study groups are considered exploratory in nature. As such, no adjustments will be required for multiple comparisons.

12.3.4. Subgroup Analyses

All exploratory analyses, including any subgroups that require additional consideration, will be discussed in the SAP, if necessary.

12.3.5. Missing Data

Standard procedures will be used to ensure that the data are as complete and accurate as possible. Due to the exploratory nature of this study, all descriptive summaries will be based upon all available data, and no imputation will be done.

12.4. Procedure for Documenting Deviations from the Planned Analyses

The principal features of the design of this study and of the plan for statistical analysis of the data are outlined in this protocol. Additional details will be included in the SAP before initiating analyses. Any changes to that plan will be documented in the final clinical study report and will be approved by BARDA before being initiated.

13. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

13.1. Inspection of Records

BARDA, Rho, the IRB, and regulatory authorities will be allowed to conduct site visits to the investigational facilities for the purpose of monitoring, inspecting, or auditing any aspect of the study.

The investigator agrees to allow BARDA, Rho, the IRB, and regulatory authorities to inspect the clinical facilities, including the study IP storage area, and all documentation relating to the study, including but not limited to all source documents, eCRFs, IRB submissions and approvals, study IP accountability logs, study IP temperature monitoring logs, regulatory documents, and correspondence.

The investigator also agrees to promptly notify BARDA and Rho of any audits or inspections scheduled by any regulatory authority and promptly forward copies of any audit or inspection reports received.

13.2. Institutional Review Board

A copy of the protocol, informed consent forms (ICFs), subject diary, any other subject facing documents, and any proposed advertising/recruitment materials will be submitted to the central IRB for written approval. Initial IRB approval and all materials approved by the IRB for this study, including the subject consent form and recruitment materials, must be maintained by the investigator and made available for inspection.

14. QUALITY CONTROL AND QUALITY ASSURANCE

Quality assurance includes all the planned and systematic actions that are established to ensure that the clinical study is performed and the data are generated, documented (recorded), and reported according to ICH GCP and local/regional regulatory standards.

A quality assurance representative from BARDA (or designee), who is independent of and separated from routine monitoring, may periodically arrange inspections/audits of the clinical study by reviewing the data obtained and procedural aspects. These inspections may include on-site inspections/audits and source data checks. Direct access to source documents is required for the purpose of these periodic inspections/audits.

14.1. Data Quality Assurance

Before enrolling any subjects in this study, BARDA personnel and the investigator will review the protocol, BioThrax package insert, AV7909 investigator's brochure, the eCRFs and instructions for their completion, the procedure for obtaining informed consent, and the procedure for reporting AEs, SAEs, MAAEs, and PIMMCs.

As part of the responsibilities assumed by participating in the study, the investigator agrees to maintain adequate source documents for the subjects treated as part of the research under this protocol. In addition, the investigator agrees to provide access to those records to BARDA's study monitor and auditor. Study monitors will verify information in the eCRFs against the source documents.

Data collected at the study site will be entered accurately and contemporaneously by study staff into Medidata RAVE, a 21CFR11-compliant, internet-based, remote data entry system, which is backed up nightly with backup tapes saved in a secure, off-site location. Data will be provided using the subject's unique identification number, not name or initials; Rho will not collect personally identifying information such as the subject's name or social security number. Subjects will provide demographic information such as race, ethnicity, and birth date. All elements of data entry (e.g., time, date, verbatim text, and the person performing the data entry) will be recorded within the RAVE system's audit trail to allow all data changes in the database to be monitored and maintained in accordance with federal regulations. Data collected by the laboratories will be transferred electronically directly from the laboratory to Rho using standard secure data transfer procedures. The analysis datasets will incorporate data from both sources. Data collected by Rho will be held in the strictest confidence, and are protected from access that could reveal personally identifying information about any subject in the study.

Clinical data management and data cleaning procedures (i.e., resolving errors and inconsistencies in the data) will be performed in accordance with applicable Rho and/or BARDA standards and validation plans to ensure the integrity of the data. Adverse events (including SAEs, MAAEs, and PIMMCs) and concomitant medication terms will be coded using the Medical Dictionary for Regulatory Activities and the World Health Organization Drug dictionaries, respectively.

At the interim analysis, the study database (all data through Day 64) will be monitored and cleaned per the DMP. For the final analysis, the study database will be monitored, cleaned, and locked per the DMP.

The investigator will sign each subject's eCRF to confirm accuracy of the data recorded for the Day 64 interim analysis and at the end of the study. After the end of study database lock, each study site will receive an electronic copy of all of their site-specific eCRF data as entered into Medidata RAVE for the study, including full discrepancy and audit history. Additionally, all of the study's analysis datasets will be sent to BARDA electronically for storage. Rho will maintain a duplicate copy for its records.

14.2. Study Monitoring

According to ICH GCP guidelines, the sponsor of the study is responsible for ensuring the proper conduct of the study with regard to protocol adherence and validity of data recorded on the eCRFs. This study will be monitored to ensure the maintenance of complete, accurate, legible, well-organized, and easily retrievable data. In addition, the study monitor will explain and interpret for the investigator all regulations applicable to the clinical evaluation of an investigational product as documented in ICH guidelines.

It is the study monitor's responsibility to inspect the eCRFs and source documentation directly throughout the study to protect the rights of the subjects; to verify adherence to the protocol; to verify completeness, accuracy, and consistency of the data; to perform study IP accountability; and to confirm adherence of study conduct to any local regulations. Details will be outlined in the Clinical Site Monitoring Plan.

14.3. Protocol Deviations

14.3.1. Protocol Deviation Definition

A protocol deviation is any noncompliance with the IRB-approved study protocol, ICH GCP guidelines, protocol-specific operational documents, or applicable regulatory requirements. Any deviation that affects the rights or safety of the subject or the integrity of the data will be considered a major protocol deviation. Prospective permission to deviate from protocol requirements (i.e., "study waivers") will not be granted for this study.

14.3.2. Reporting and Managing Deviations

The investigator has the responsibility to identify, document, and report deviations. Protocol deviations may also be identified during site monitoring visits or during other forms of study conduct review. All deviations, regardless of the cause, must be documented on the appropriate eCRF, which will document at a minimum the date the deviation occurred, the date it was identified, a description of the deviation, and documentation of a corrective/preventative action. In addition, the investigator will report noncompliance to the IRB, as applicable.

Rho and/or BARDA may request discussion with the site investigator to determine the effect of any major protocol deviation on a study subject and his/her further study participation, the effect of the deviation on the overall study, and corrective actions.

15. ETHICS

15.1. Ethics Review

Before study initiation, the protocol, the informed consent documents, the subject diary, any other subject facing documents, and any proposed advertising/recruitment materials will be reviewed and approved by the IRB centrally; in addition, each individual site will be reviewed and approved by the IRB prior to the shipment of study IP to and the activation of each individual site. Any amendments to those documents will be submitted to the IRB (following approval by BARDA) and must be approved by the IRB before they are implemented at the sites. Protocol documents must be re-approved by the IRB annually. Only institutions holding a current US Federal Wide Assurance issued by the Office for Human Research Protections at HHS may participate in the study.

The investigator will promptly report all unanticipated problems involving risks to subjects to the IRB, as applicable. The investigator will not make any changes to the research conduct without BARDA and IRB approval, except where necessary to eliminate apparent immediate hazards to the subjects.

The investigator will provide progress reports to the IRB as required by the IRB. The investigator will provide a final report to the IRB after completion of participation in the study.

15.2. Ethical Conduct of the Study

The investigator should conduct the study in accordance with this protocol, the Declaration of Helsinki, current ICH GCP guidelines, and 21 CFR Part 50 (Protection of Human Subjects) and Part 56 (IRBs).

15.3. Written Informed Consent

The informed consent document template, including site-specific versions, will be approved by the IRB. The investigator is responsible for ensuring that the subject fully understands the nature and purpose of the study during the informed consent process. Information should be given in both oral and written form. Given that this study includes a vulnerable elderly population, subject comprehension will be assessed by administering an "Assessment of Understanding Questionnaire" during the consent process. No subject should be obliged to participate in the study. The information must make clear that refusal to participate in the study or withdrawal from the study at any stage is without any prejudice to the subject's subsequent care. Subjects must be allowed sufficient time to inquire about the details of the study and to decide whether they wish to participate. Written informed consent will be obtained before the subject undergoes any study procedures.

The subject must be made aware of and give consent to direct access to his/her source medical records by study monitors, auditors, the IRB, and regulatory authorities. The subject should be informed that such access will not violate subject confidentiality or any applicable regulations. The subject should also be informed that he/she is authorizing such access by signing the ICF.

The investigator will retain the original signed informed consent, and each subject will be given a signed copy to keep for his/her records.

Using the ICF, subjects who consent to participate in this study will also be asked to consent to the following:

- Permission to have a photograph of a visible injection site abnormality for the purpose of medical management and safety evaluations.
- Permission to be contacted with a request to participate in future clinical studies of subjects who have been vaccinated against *B. anthracis*, such as longer interval prime-boost studies.
- Permission to store any leftover serum for future investigations unrelated to this study. Leftover serum will be stored indefinitely. Subjects who provide consent may withdraw that consent for future investigations during their participation in the study by contacting the study doctor identified on the consent form. After their participation in the study has ended, subjects can contact BARDA's Chief Medical Officer (202-260-1200). Leftover serum will be destroyed, but any data that have already been collected from analysis of leftover serum will remain a part of the research database.

16. DATA HANDLING AND RECORDKEEPING

16.1. Confidentiality

Each subject will be assigned a unique identification number and these numbers rather than names or other personal health information will be used to collect, store, and report subject information. All biological samples will be labeled with a unique identification number rather than names or other personal health information. Site staff will not transmit documents containing personal health identifiers to BARDA or its representatives. Data reported in medical journals or scientific meetings will be presented in aggregate for subjects as a whole. No individual subject will be identified in any way.

16.2. Retention of Records

The investigator should retain all documentation relating to the study (including but not limited to ICFs, source documentation, study IP records, eCRFs, and essential documents) for a period of at least 2 years after the last marketing application approval or, if not approved, 2 years following the discontinuance of the test article for investigation.

If this requirement differs from any local regulations, the local regulations will take precedence unless the local retention policy is less than 2 years.

At study closure, the investigator must inform BARDA, or designee, of the long-term storage location of the study's records. Following study closure, the investigator must inform BARDA if that location changes (e.g., the investigator leaves the institution where the study was conducted).

No study records will be destroyed without prior authorization from BARDA.

17. PUBLICATION POLICY

BARDA will be responsible for publication activities and will work with the investigators to define the manuscript/presentation development process, the number and order of authors, the publication/scientific meeting to which it will be submitted, and other related issues. BARDA has final approval authority over all such issues.

Data are the property of BARDA and cannot be published without prior authorization from BARDA, but data and publication thereof will not be unduly withheld.

The Assistant Secretary for Preparedness and Response (ASPR) Public Access Plan[15] and the National Institutes of Health Public Access Policy[16] will apply to this study. The ASPR-funded investigators will be required to submit an electronic version of final, peer-reviewed manuscripts resulting from this study to the National Library of Medicine's PubMed Central upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication.

18. REFERENCES

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APPENDIX 1. SCHEDULE OF ASSESSMENTS

Study Visit	**Screen-ing**	**V1**	**V2[a]**	**V3**	**V4[b]**	**C1[c,b]**	**V5[b]**	**V6[b]**	**C2[c,b]**	**V7[b]**	**V8[b]**	**V9**	**V10**	**V11**	**V12[d]**	**C3[c]**	**V13**	**ET[d]**	**UV**
Study Day	**D-1 to D-14**	**D1**	**D3 ±1 D**	**D8 +2 D**	**D15 -1 to +3 D**	**D17 ±1 D**	**D22 +2 D**	**D29 -1 to +3 D**	**D31 ±1 D**	**D36 +2 D**	**D43 -2 to +1 D**	**D50 ±2 D**	**D64 ±3 D**	**D85 ±3 D**	**D181 ±14 D**	**D366 ±7 D**	**D394 ±14 D**	**N/A**	**N/A**
Visit window based on actual Vaccination Day	**N/A**	**N/A**	**V1+2 ±1 D**	**V1+7 +2 D**	**V1+14 -1 to +3 D**	**V4+2 ±1 D**	**V4+7 +2 D**	**V4+14 -1 to +3 D**	**V6+2 ±1 D**	**V6+7 +2 D**	**V6+14 -2 to +1 D**	**V1+49 ±2 D**	**V1+63 ±3 D**	**V1+84 ±3 D**	**V1+180 ±14 D**	**V1+365 ±7 D**	**V6+365 ±14 D**	**N/A**	**N/A**
Procedure																			
Obtain Informed Consent	X																		
Inclusion/Exclu-sion Criteria Assessment[e]	X	X[f]			X[f]			X[f]											
Urine Pregnancy Test[g]	X	X[f]			X[f]			X[f]											
Physical Exam, Medical History, Height, and Weight	X																		
Symptom-Directed PE		X	X	X	X		X	X		X		X	X					X	X
Vital Signs[h]	X	X[f]	X	X	X[f]		X	X[f]		X		X	X					X	X
Post-vaccination Vital Signs[i]		X			X			X											
HIV/HBV/HCV, HbA1C, and urine drug screen[j, k]	X																		
ECG	X									X							X		
CBC and Chemistry[l]	X			X			X			X								X[m]	X[m]
hsCRP[a]		X[f]	X																
Autoantibody[n]		X[f]											X					X	
Urinalysis[o]	X			X			X			X								X[m]	X[m]

Study Visit	**Screen-ing**	**V1**	**V2[a]**	**V3**	**V4[b]**	**C1[c,b]**	**V5[b]**	**V6[b]**	**C2[c,b]**	**V7[b]**	**V8[b]**	**V9**	**V10**	**V11**	**V12[d]**	**C3[c]**	**V13**	**ET[d]**	**UV**
Study Day	**D-1 to D-14**	**D1**	**D3 ±1 D**	**D8 +2 D**	**D15 -1 to +3 D**	**D17 ±1 D**	**D22 +2 D**	**D29 -1 to +3 D**	**D31 ±1 D**	**D36 +2 D**	**D43 -2 to +1 D**	**D50 ±2 D**	**D64 ±3 D**	**D85 ±3 D**	**D181 ±14 D**	**D366 ±7 D**	**D394 ±14 D**	**N/A**	**N/A**
Visit window based on actual Vaccination Day	**N/A**	**N/A**	**V1+2 ±1 D**	**V1+7 +2 D**	**V1+14 -1 to +3 D**	**V4+2 ±1 D**	**V4+7 +2 D**	**V4+14 -1 to +3 D**	**V6+2 ±1 D**	**V6+7 +2 D**	**V6+14 -2 to +1 D**	**V1+49 ±2 D**	**V1+63 ±3 D**	**V1+84 ±3 D**	**V1+180 ±14 D**	**V1+365 ±7 D**	**V6+365 ±14 D**	**N/A**	**N/A**
Immunogenicity Profile[p]		X[f]		X			X	X[f]		X	X	X	X	X	X		X	X[q]	X[q]
Randomization		X																	
Vaccination and Post-vaccination Observation		X			X			X											
Injection Site Evaluation[r]		X	X	X	X		X	X		X	X								
Dispense and or Review Diary[s]		X	X	X	X	X	X	X	X	X	X	X						X[t]	X[t]
AE/SAE/MAAE/PIMMC assessment		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Prior and Concomitant Medications[u]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

AE = adverse event; ANA = anti-nuclear antibody; C = Contact; CBC = complete blood count; D = day; dsDNA = double-stranded deoxyribonucleic acid; eCRF = electronic case report form; ECG = electrocardiogram; ET = early termination; HbA1C = hemoglobinA1C; hsCRP = high-sensitivity C-reactive protein; Ig = immunoglobulin; MAAE = medically attended adverse event; N/A = not applicable; PA = protective antigen; PE = physical examination; PIMMC = potentially immune-mediated medical condition; SAE = serious adverse event; TNA = toxin neutralization antibody; UV = unscheduled visit; V = visit

[a] Only for subjects ≥66 years of age.

[b] If a subject discontinues the vaccination series only the following procedures should be completed: 1) Visit 4 and Visit 6 – Symptom-Directed PE, Vital Signs, Review Diary, AE/SAE/MAAE/PIMMC assessment, Concomitant Medications, and Immunogenicity Profile (Visit 6 only); 2) Contact 1 and Contact 2 – AE/SAE/MAAE/PIMMC assessment and Concomitant Medications; 3) Visit 5 and Visit 7, if a subject was not vaccinated at Visit 4 and/or Visit 6 – Symptom-Directed PE, Vital Signs, AE/SAE/MAAE/PIMMC assessment, Concomitant Medications, and Immunogenicity Profile; 4) Visits 5, 6, 7, and 8 – Injection site evaluation for the previous vaccination will be performed as applicable. Subjects who become pregnant during the study will only be followed for safety as presented above, and will not provide blood for the Immunogenicity Profile.

[c] Telephone visit

[d] Early Termination Visit procedures are to be performed for subjects who discontinue study participation prior to Day 64. If subjects are unable to return to the site for an ET visit, an attempt should be made to assess AE/SAE/MAAE/PIMMC and pregnancy information via telephone at a minimum.

[e] If a subject has a fever at a vaccination visit, they can be re-assessed for eligibility within the visit window (+3 Days) and vaccinated for the 2nd or 3rd IP dose if eligibility criteria are met.

[f] Prior to vaccination

[g] Only in women of childbearing potential; onsite urine pregnancy test must be performed at Screening and within 24 hours prior to each vaccination, and pregnancy test results must be final and negative prior to vaccination.

[h] Vital signs include systolic and diastolic blood pressure (sitting for at least 5 minutes), pulse oximeter reading, heart rate, respiratory rate, and oral temperature; obtain vital signs before vaccination at Visits 1, 4, and 6. Obtain vital signs prior to any blood draws.

[i] Vital signs will be taken 30 minutes (±5 minutes) and 1 hour (±5 minutes) post-vaccination prior to discharge.

[j] Includes HIV antibody, hepatitis B surface antigen, and hepatitis C antibody.

[k] Urine drug screen includes tests for amphetamines, barbiturates, benzodiazepines, cocaine, tetrahydrocannabinol, methylenedioxymeth-amphetamine, opiates, oxycodone, phencyclidine, and propoxyphene.

[l] Complete blood count with differential and chemistry including alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine, glucose (random, serum), and total bilirubin.

[m] If clinically indicated in the investigator's judgment.

[n] Serum samples will be collected and frozen for potential testing of ANA, anti-dsDNA, and rheumatoid factor titers if needed.

[o] Urinalysis parameters include appearance, color, pH, specific gravity, ketones, protein, glucose, bilirubin, nitrite, urobilinogen and occult blood.

[p] Immunogenicity profile includes serum for TNA and anti-PA IgG determinations; obtain sample prior to each vaccination on vaccination days.

[q] Only collect immune profile sample at ET Visit or UV if within the acceptable window for the next expected study visit.

[r] The Dose 1 injection site will be examined at Visit 1 (Day 1) at 1 hour (±5 minutes) post-vaccination, Visit 2 (Day 3), Visit 3 (Day 8), and at Visit 4 (Day 15 prior to Dose 2 vaccination). The Dose 2 injection site and the Dose 1 injection site (contralateral arm, if applicable) will be examined at Visit 4 (Day 15) at 1 hour (±5 minutes) post-vaccination, Visit 5 (Day 22), and Visit 6 (Day 29 prior to Dose 3 vaccination). The Dose 3 injection site and the Dose 2 injection site (contralateral arm, if applicable) will be examined at Visit 6 (Day 29) at 1 hour (±5 minutes) post-vaccination, Visit 7 (Day 36), and Visit 8 (Day 43). If a visible injection site abnormality is observed, the abnormality will be documented with a photograph if the subject consented.

[s] Diary cards will be dispensed at Visits 1, 4, and 6. Diary cards 1A, 2A, and 3A will be used to collect solicited and unsolicited local and systemic symptoms, concomitant medication changes, and temperatures within 8 days after each vaccination, inclusive of the vaccination day. Diary cards 1B, 2B, and 3B will be used to collect unsolicited local and systemic symptoms on days 8-15 after Dose 1 and 2 and days 8-21 after Dose 3. Study site staff will train each subject at Visit 1 to take their own temperature in front of site staff to confirm correct measurement before leaving the clinic and will train subjects on how to measure injection site reactions.

[t] If applicable, according to the protocol period, collect and review diary card with subject.

[u] Prior medications taken in the past 6 months and vaccinations within 30 days will be assessed at Screening to determine eligibility. Prior medications and vaccinations administered within 30 days of Screening will be recorded on the subject's eCRF. All concomitant medications used by the subject from the time of consent until Visit 9 (Day 50) will be recorded in the subject's eCRF. Following Visit 9 (Day 50), concomitant medications will only be recorded if they are used for the treatment of an MAAE, SAE, or PIMMC. Nonstudy vaccines received through Visit 10 (Day 64) will also be recorded in the subject's eCRF.

APPENDIX 2. TABLES FOR CLINICAL AND LABORATORY ABNORMALITIES

The tables for clinical and laboratory abnormalities are presented in Table 8 and Table 9, respectively, according to the FDA Guidance for Industry: *Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (September 2007).*

Table 8: Tables for Clinical Abnormalities

Local Reaction to Injectable Product	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever >24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	Emergency room visit or hospitalization
Erythema/redness[a]	2.5-5 cm	5.1-10 cm	>10 cm	Necrosis or exfoliative dermatitis
Induration/swelling[b]	2.5-5 cm and does not interfere with activity	5.1-10 cm or interferes with activity	>10 cm or prevents daily activity	Necrosis

[a] In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
[b] Induration/swelling should be evaluated and graded using the functional scale as well as the actual measurement.

Vital Signs[a]	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Fever (°C)[b] (°F)[b]	38.0-38.4 100.4-101.1	38.5-38.9 101.2-102.0	39.0-40 102.1-104	>40 >104
Tachycardia – beats per minute	101-115	116-130	>130	ER visit or hospitalization for arrhythmia
Bradycardia – beats per minute[c]	50-54	45-49	<45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) – mm Hg	141-150	151-155	>155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) – mm Hg	91-95	96-100	>100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85-89	80-84	<80	ER visit or hospitalization for hypotensive shock

Vital Signs[a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Respiratory rate – breaths per minute	17-20	21-25	>25	Intubation

ER = emergency room
[a] Subject should be at rest for all vital sign measurements.
[b] Oral temperature; no recent hot or cold beverages or smoking.
[c] When resting heart rate is between 60 to 100 beats per minute. Use clinical judgment when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Systemic (General)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Nausea/vomiting	No interference with activity or 1-2 episodes/24 hours	Some interference with activity or >2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhea	2-3 loose stools or <400 g/24 hours	4-5 stools or 400-800 g/24 hours	6 or more watery stools or >800 g/24 hours or requires outpatient IV hydration	ER visit or hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever >24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization

ER = emergency room; IV = intravenous

Table 9: Tables for Laboratory Abnormalities

Serum[a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)[b]
Sodium – hyponatremia mEq/L	132-134	130-131	125-129	<125
Sodium – hypernatremia mEq/L	144-145	146-147	148-150	>150
Potassium – hyperkalemia mEq/L	5.1-5.2	5.3-5.4	5.5-5.6	>5.6
Potassium – hypokalemia mEq/L	3.5-3.6	3.3-3.4	3.1-3.2	<3.1

Serum[a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)[b]
Glucose – hypoglycemia mg/dL	65-69	55-64	45-54	<45
Glucose – hyperglycemia Fasting - mg/dL Random - mg/dL	 100-110 110-125	 111-125 126-200	 >125 >200	Insulin requirements or hyperosmolar coma
Blood urea nitrogen mg/dL	23-26	27-31	>31	Requires dialysis
Creatinine mg/dL	1.5-1.7	1.8-2.0	2.1-2.5	>2.5 or requires dialysis
Calcium-hypocalcemia mg/dL	8.0-8.4	7.5-7.9	7.0-7.4	<7.0
Calcium-hypercalcemia mg/dL	10.5-11.0	11.1-11.5	11.6-12.0	>12.0
Magnesium-hypomagnesemia mg/dL	1.3-1.5	1.1-1.2	0.9-1.0	<0.9
Phosphorus – hypophosphatemia mg/dL	2.3-2.5	2.0-2.2	1.6-1.9	<1.6
CPK - mg/dL	1.25-1.5 × ULN	1.6-3.0 × ULN	3.1-10 × ULN	>10 × ULN
Albumin – hypoalbuminemia g/dL	2.8-3.1	2.5-2.7	<2.5	–
Total protein – hypoproteinemia g/dL	5.5-6.0	5.0-5.4	<5.0	–
Alkaline phosphate – increase by factor	1.1-2.0 × ULN	2.1-3.0 × ULN	3.1-10 × ULN	>10 × ULN
Liver function tests – ALT, AST increase by factor	1.1-2.5 × ULN	2.6-5.0 × ULN	5.1-10 × ULN	>10 × ULN
Bilirubin – when accompanied by any increase in liver function test increase by factor	1.1-1.25 × ULN	1.26-1.5 × ULN	1.51-1.75 × ULN	>1.75 × ULN
Bilirubin – when liver function test is normal; increase by factor	1.1-1.5 × ULN	1.6-2.0 × ULN	2.0-3.0 × ULN	>3.0 × ULN
Cholesterol mg/dL	201-210	211-225	>226	-
Pancreatic enzymes – amylase, lipase	1.1-1.5 × ULN	1.6-2.0 × ULN	2.1-5.0 × ULN	>5.0 × ULN

ALT = alanine aminotransferase; AST = aspartate aminotransferase; CPK = creatine phosphokinase; ULN = upper limit of normal.

[a] The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

[b] The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example, a low sodium value that falls within a Grade 3 parameter (125-129 mE/L) should be recorded as a Grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.

Hematology[a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (female) – g/dL	11.0-12.0	9.5-10.9	8.0-9.4	<8.0
Hemoglobin (female) change from baseline value – g/dL	Any decrease – 1.5	1.6-2.0	2.1-5.0	>5.0
Hemoglobin (male) – g/dL	12.5-13.5	10.5-12.4	8.5-10.4	<8.5
Hemoglobin (male) change from baseline value – g/dL	Any decrease – 1.5	1.6-2.0	2.1-5.0	>5.0
WBC increase – cell/mm^3	10,800-15,000	15,001-20,000	20,001-25,000	>25,000
WBC decrease – cell/mm^3	2,500-3,500	1,500-2,499	1,000-1,499	<1,000
Lymphocytes decrease – cell/mm^3	750-1 000	500-749	250-499	<250
Neutrophils decrease – cell/mm^3	1,500-2,000	1,000-1,499	500-999	<500
Eosinophils – cell/mm^3	650-1 500	1501-5 000	>5 000	Hypereosinophilic
Platelets decrease – cell/mm^3	125,000-140,000	100,000-124,000	25,000-99,000	<25,000
PT – increase by factor	1.0-1.10 × ULN	1.11-1.20 × ULN	1.21-1.25 × ULN	>1.25 x ULN
PTT – increase by factor	1.0-1.2 × ULN	1.21-1.4 × ULN	1.41-1.5 × ULN	>1.5 x ULN
Fibrinogen increase – mg/dL	400-500	501-600	>600	–
Fibrinogen decrease – mg/dL	150-200	125-149	100-124	<100 or associated with gross bleeding or disseminated intravascular coagulation

PPT = partial thromboplastin time; PT = prothrombin time; ULN = upper limit of normal; WBC = white blood cell.
[a] The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

Urine[a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Protein	Trace	1+	2+	Hospitalization or dialysis
Glucose	Trace	1+	2+	Hospitalization or hyperglycemia
Blood (microscopic) – red blood cells per high power field	1-10	11-50	>50 and/or gross blood	Hospitalization or packed red blood cells transfusion

[a] The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

APPENDIX 3. LIST OF POTENTIALLY IMMUNE-MEDIATED MEDICAL CONDITIONS

Gastrointestinal disorders

- Celiac disease
- Crohn's disease
- Ulcerative colitis
- Ulcerative proctitis
- Microscopic colitis
- Autoimmune pancreatitis

Hematologic disorders

- Pernicious anemia
- Autoimmune thrombocytopenia
- Autoimmune hemolytic anemia
- Autoimmune neutropenia
- Autoimmune pancytopenia

Liver disorders

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

Metabolic/Endocrine diseases

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

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 (calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia) 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, radiculomyelitis
- 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
- Lambert-Eaton syndrome

Skin disorders

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

- Stevens-Johnson syndrome

Vasculitides

- Large vessel vasculitis including giant cell arteritis such as Takayasu's arteritis and temporal arteritis
- Medium sized and/or small vessel 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 antineutrophil cytoplasmic antibody (ANCA)-positive vasculitis (type unspecified), Henoch-Schonlein purpura, Behcet's syndrome, leukocytoclastic vasculitis

Others

- Antiphospholipid syndrome
- Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis)
- Autoimmune myocarditis/cardiomyopathy
- Goodpasture syndrome
- Idiopathic pulmonary fibrosis
- Raynaud's phenomenon
- Sarcoidosis
- Sjögren's syndrome
- Uveitis