

BLA Clinical Review Memorandum

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Division / Office	DVRPA/OVRR
Priority Review (Yes/No)	No
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Supervisory Concurrence	Meghan Ferris, M.D. M.P.H. Andrea N. Hulse, M.D.
Applicant	Emergent Product Development Gaithersburg Inc.
Established Name	Anthrax Vaccine Adsorbed, Adjuvanted
Trade Name	Cyfundus
Pharmacologic Class	Vaccine
Formulation(s), including Adjuvants, etc.	Anthrax Vaccine Adsorbed plus CpG 7909 Adjuvant
Dosage Form(s) and Route(s) of Administration	0.5 mL administered intramuscularly (IM)
Dosing Regimen	Week 0 and Week 2 (2-dose regimen)
Indication(s) and Intended Population(s)	Post-exposure prophylaxis (PEP) of disease following suspected or confirmed exposure to <i>Bacillus anthracis</i> in persons 18 through 65 years of age when administered in conjunction with the recommended antibacterial regimen.
Orphan Designated (Yes/No)	Yes

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GLOSSARY

200 LD ₅₀	50% lethal dose by 200-fold
ACIP	Advisory Committee on Immunization Practices
ADAE	adverse events analysis
ADaM	Analysis Data Model
ADFACE	analysis dataset findings about clinical events
AE	adverse event
AESI	adverse event of special interest
ALC	absolute lymphocyte count
ALT	alanine aminotransferase
AUC _{0-12h}	area under the concentration-time curve from 0 to 12 hours
AUC _{0-∞}	area under the concentration-time curve extrapolated to infinity
AUMC	area under the first moment curve
AV7909	Anthrax Vaccine Adsorbed plus CpG 7909 adjuvant
AVA	Anthrax Vaccine Adsorbed
<i>B. anthracis</i>	<i>Bacillus anthracis</i>
BARDA	Biomedical Advanced Research and Development Authority
BIMO	Bioresearch Monitoring Program
BLA	Biologics License Application
BMI	body mass index
BUN	blood urea nitrogen
CBER	Center for Biologics Evaluation and Research
CDC	Centers for Disease Control and Prevention
CDER	Center for Drug Evaluation and Research
CE	clinical event
CFR	Code of Federal Regulations
CI	confidence interval
CMC	Chemistry, Manufacturing, and Controls
CRO	contract research organization
C _{max}	maximum observed concentration
CSR	clinical study report
CT	computed tomography
CV	coefficient of variation
DBPAP	Division of Bacterial Polysaccharides and Allergenic Products
DSMB	Data Safety Monitoring Board
DPV	Division of Pharmacovigilance
dsDNA	double-stranded deoxyribonucleic acid antibody
eCRF	electronic case report form
ED ₅₀	50% neutralization of lethal toxin cytotoxicity
EF	edema factor
Emergent	Emergent Product Development Gaithersburg Inc.
EMR	electronic medical records
ET	edema toxin
EUA	emergency use authorization
EWV	Early Withdrawal Visit
FDA	Food and Drug Administration
FSH	follicle-stimulating hormone
GCP	good clinical practice
GLP	good laboratory practice
GMR	geometric mean ratio

GMT	geometric mean titer
GUP	general use prophylaxis
HBsAg	hepatitis B surface antigen
HIV	human immunodeficiency virus
ICF	informed consent form
ID	intra dermal
IgG	immunoglobulin G
IM	intramuscular
IND	Investigational New Drug
IP	investigational product
IR	Information Request
ISE	Integrated Summary of Efficacy
ISS	Integrated Summary of Safety
ITT	intent-to-treat
IV	intravenous
IxRS	interactive voice and/or web response system
K_{el}	apparent elimination rate constant
LB	lower bound
LF	lethal factor
LLOQ	lower limit of quantitation
LMP	last menstrual period
LT	lethal toxin
MA	Major Amendment
MCM	medical counter measure
MDR	multi-drug resistant
MedDRA	Medical Dictionary for Regulatory Activities
MM	Medical Monitor
MRT	mean residence time
MTD	material threat determination
NF_{50}	50% neutralization factor
NIAID	National Institute of Allergy and Infectious Diseases
NHP	non-human primate
OCBQ	Office of Compliance and Biologics Quality
ODN	oligodeoxynucleotide
OTC	over-the-counter
PA	protective antigen
PD	pharmacodynamics
PE	physical examination
PEP	post-exposure prophylaxis
PI	principal investigator
PK	pharmacokinetics
PMR	postmarketing requirement
PO	per os (by mouth)
PP	per protocol
PPROM	preterm premature rupture of membranes
PREA	Pediatric Research Equity Act
PT	preferred term
PVE	predicted vaccine efficacy
PVP	pharmacovigilance plan
R_{abs}	rate of absorption
REMS	Risk Evaluation and Mitigation Strategy

RF	rheumatoid factor
SAE	serious adverse event
SAP	statistical analysis plan
sBLA	supplemental Biologics License Application
SC	subcutaneous
SD	standard deviation
SDTM	Study Data Tabulation Model
SNS	Strategic National Stockpile
SOC	System Organ Class
SUSAR	suspected unexpected serious adverse reaction
TEAE	treatment-emergent adverse event
TLR9	Toll-like receptor 9
T _{max}	time of maximum observed concentration
TNA	toxin-neutralizing antibody
T _{1/2}	half-life
TSH	thyroid-stimulating hormone
ULN	upper limit of normal
US	United States
USPI	United States Prescribing Information (package insert)
VE	vaccine efficacy
VRBPAC	Vaccines and Related Biological Products Advisory Committee
VS	vital signs
WBC	white blood cell
WOCBP	women of childbearing potential

1. EXECUTIVE SUMMARY

Emergent Product Development Gaithersburg, Inc. (also referred to as Emergent or the Applicant) submitted a Biologics License Application (BLA) to support approval of Anthrax Vaccine Adsorbed plus CpG 7909 adjuvant (AV7909) for post-exposure prophylaxis (PEP) of disease following suspected or confirmed exposure to *Bacillus anthracis* (*B. anthracis*) in persons 18 through 65 years of age when administered in conjunction with the recommended antibacterial regimen.

AV7909 is an anthrax vaccine that consists of AVA bulk drug substance that is similar in composition and manufacturing process to BioThrax combined with CpG 7909. AV7909 is not currently licensed or authorized in any country. BioThrax (AVA; anthrax vaccine adsorbed) is the anthrax vaccine currently Food and Drug Administration (FDA) approved for PEP when administered in conjunction with the recommended antibacterial regimen.¹

CpG 7909 is an immunostimulatory synthetic oligodeoxynucleotide (ODN) that functions as an adjuvant by activation of Toll-like receptor 9 (TLR9). It is designed to induce both innate immunity and an enhanced antigen-specific antibody response.

It is unethical to conduct clinical studies in which humans are intentionally exposed to *B. anthracis*, and the unpredictable incidence of disease makes it difficult to conduct field studies. Thus, the Applicant seeks approval of AV7909 for PEP under the Animal Rule (21 Code of Federal Regulations [CFR] 601 Subpart H for Biologics, "Approval of Biological Products when Human Efficacy Studies are not Ethical or Feasible"). Using the Animal Rule to license vaccines to protect against anthrax was recommended by the Vaccines and Related Biological Products Advisory Committee (VRBPAC) in 2010² and was used to license BioThrax for PEP against anthrax disease under supplemental Biologics License Application (sBLA) STN 103821/5344.^{3,4,5}

The ability to use the Animal Rule pathway required that AV7909 elicit an immune response in humans comparable to that in animals protected by the vaccine after *B. anthracis* spore challenge. The pathogenic mechanisms resulting in inhalational anthrax are well characterized and shown to closely resemble the human disease in both the rabbit and non-human primate (NHP; cynomolgus macaque) aerosol challenge models.² Given that anthrax disease in these animals mimics human anthrax disease, they were selected as the pivotal animal models supporting BioThrax PEP licensure. Passive transfer animal studies performed with purified human immune globulin generated by vaccination with BioThrax in support of its PEP licensure confirmed the ability of neutralizing anti-protective antigen (PA) antibodies to protect animals against death from anthrax disease.³

To assess the effectiveness of the proposed AV7909 human dose, immune responses associated with survival in animals were bridged to human immunogenicity data to infer clinical benefit. The toxin neutralizing antibody (TNA) assay which measures functional antibody that binds anthrax toxin² was used to determine this threshold of protection for AV7909. A validated pan-species high-throughput TNA assay was used to assess neutralizing antibody levels induced by AV7909 in all non-clinical and clinical studies.

In three guinea pig studies and three NHP studies, groups of animals were immunized with dilutions of AV7909 and were challenged on Day 28 or Day 70 with aerosolized *B. anthracis* spores. Neutralizing antibody (TNA) titers were expressed as a ratio, or 50% neutralization factor (NF₅₀), relative to a reference serum from BioThrax vaccinated subjects.

A 70% probability of survival was associated with TNA NF₅₀ titers ranging from 0.063 to 0.081 in the guinea pig studies and from 0.107 to 0.262 in the NHP studies. In previous BioThrax studies, groups of rabbits or NHPs were immunized on Days 0 and 28 with dilutions of BioThrax or placebo and challenged on Day 70 with aerosolized *B. anthracis* spores. A pre-exposure TNA NF₅₀ level of 0.56 corresponded to a 70% probability of survival in rabbits, and a pre-exposure TNA NF₅₀ level of 0.29 corresponded to a 70% probability of survival in NHPs. The Applicant selected the most conservative protective target, the NF₅₀ threshold of 0.56—derived from the pivotal BioThrax rabbit PEP study (Study 646-N107247), as the basis for the primary clinical immunogenicity endpoint in the pivotal AV7909 Phase 3 study (Study EBS.AVA.212).

Five subsequent rabbit pre-exposure prophylaxis studies showed that TNA NF₅₀ thresholds in the range of 0.19 to 0.29 correlated with 70% rabbit survival. Logistic regression analysis of pooled study data from these BioThrax-immunized rabbits (n=632) showed a TNA NF₅₀ threshold of 0.24 was associated with a 70% probability of survival. The NF₅₀ value of 0.240 obtained with the pooled rabbit data analysis was consistent with the NF₅₀ value of 0.29 obtained in the BioThrax NHP study. Results from these five rabbit studies suggested that the original rabbit study yielding the 0.564 NF₅₀ threshold overestimated the TNA threshold level. The Applicant thus proposed using the immunized NHP TNA threshold of protection NF₅₀ level from NHP Study 844 (0.294 NF₅₀) as an acceptable bridging endpoint for a proposed Phase 3 trial co-primary endpoint, to which the Center for Biologics Evaluation and Research (CBER) agreed.

Non-clinical animal studies demonstrated that AV7909 protected a large proportion of animals from death due to inhalation anthrax in a dose-dependent manner. The TNA thresholds of protection were similar between the two animal models and were not impacted by vaccination schedule or challenge time point. These studies provide supportive animal data for AV7909 PEP licensure and support the TNF NF₅₀ thresholds selected to estimate protection in the clinical trials.

Effectiveness of the proposed AV7909 human dose was determined by the proportion of subjects who achieved the protective TNA threshold correlating with enhanced (i.e., 70%) survival in a PEP setting.

In summary, human immunogenicity data combined with animal immunogenicity and survival data, along with supportive animal post-exposure studies and animal passive immunization studies which show that antibodies alone can provide protection against anthrax, comprise the essential elements of this BLA intended to support the PEP indication of AV7909 using the Animal Rule.

This BLA included four clinical studies, all of which evaluated safety, for a total safety database of 3276 subjects who received the proposed dose and schedule of AV7909.

- Studies EBS.AVA.201 and EBS.AVA.208 were Phase 1 and 2 studies, respectively, that supported selection of the AV7909 dose and dosing regimen (AVA dose of (b) (4) combined with (b) (4) mg of CpG 7909 given intramuscularly (IM) at Weeks 0 and 2) for further development.
- Study EBS.AVA.212 was a Phase 3, safety, immunogenicity, and lot-to-lot consistency study.
- Study EBS.AVA.210 was a pharmacokinetics (PK) study evaluating the bidirectional impact of AV7909 and ciprofloxacin⁵ or doxycycline⁶ use as intended in a PEP scenario.

Summary of Study EBS.AVA.212

EBS.AVA.212 was a Phase 3, double-blind, randomized, multicenter, active-controlled (BioThrax), parallel-arm, safety, lot-to-lot consistency, and immunogenicity study conducted in healthy adults 18-65 years old. AV7909 (Lots 1-3) was administered IM on Days 1 and 15, with matching placebo given on Day 29. BioThrax (0.5 mL) was administered subcutaneously (SC) on Days 1, 15, and 29.

Two sets of primary immunogenicity endpoints were evaluated: one to establish lot consistency, the other to demonstrate immunogenicity of AV7909 at a clinically relevant time point (Day 64) using a non-inferiority comparison to BioThrax. The percentage of subjects with TNA thresholds (TNA NF₅₀ ≥0.56 and ≥0.29) that correlated with 70% survival in two appropriate animal species (rabbit and NHPs, respectively) were assessed by immunobridging of human-to-animal immune responses (the second primary immunogenicity endpoint, see below), as required for licensure under the Animal Rule. Two co-primary endpoints were assigned to each of these two immunogenicity assessments (a total of four co-primary endpoints), as follows:

1. Demonstration of lot-to-lot consistency of AV7909:
 - Geometric mean titer (GMT) Ratio of TNA NF₅₀ at Day 64
 - Lot Consistency and Immunogenicity of AV7909 Evaluated with Percentage of Subjects with TNA NF₅₀ ≥0.56 at Day 64
2. AV7909 Immunogenicity at Day 64
 - AV7909 Immunogenicity of the 3 Pooled AV7909 Lots compared to BioThrax, as Defined by the Percentage of Subjects with a TNA NF₅₀ value of ≥0.56 at Day 64
 - Comparison of the Percentage of Subjects with a TNA NF₅₀ ≥0.29, AV7909 vs. BioThrax at Day 64

The pre-specified criteria for the two AV7909 immunogenicity co-primary endpoints at Day 64 were met, thereby demonstrating both lot consistency and a protective level of immunogenicity at 7 weeks (Day 64) after IM administration of the second dose of AV7909 (Weeks 0 and 2) in healthy adults (18-65 years of age).

As pervasive errors and data discrepancies in the clinical event (CE) and adverse event (AE), and analysis dataset findings about clinical events (ADFACE) and adverse events analysis (ADAE) datasets were identified during the review and data integrity issues were identified at one of the clinical study sites (US1027) in EBS.AVA.212, reanalyses of the four co-primary immunogenicity endpoints were performed excluding site US1027 and with revised datasets. The reanalyses did not reveal any significant numerical changes in the immunogenicity results which would affect immunogenicity conclusions in the study. In summary, AV7909 met all prespecified immunogenicity success criteria, with effectiveness and lot consistency demonstrated in Study EBS.AVA.212.

Safety was assessed in 3151 AV7909 recipients in Study EBS.AVA.212. Slightly greater local reactogenicity was observed in BioThrax vaccinated subjects (91.0% frequency of any injection site reaction for BioThrax compared to 86.9% for AV7909 after the first vaccination per e-diary results) and slightly greater systemic reactogenicity was seen in AV7909-vaccinated subjects (74.1% frequency of any systemic reaction for AV7909 compared to 67.9% for BioThrax after the first vaccination per e-diary results). Injection site reactions were relatively frequent in both the AV7909 and BioThrax groups (i.e., tenderness, pain, and myalgia). Most reactions in

AV7909-vaccinated subjects were Grade 1 or 2. There were no Grade 4 local or systemic reactions reported in EBS.AVA.212.

The most common treatment emergent adverse events (TEAEs) were related to injection site reactions and comprised the following (in decreasing order of frequency): injection site pain (AV7909: 4.6%; BioThrax: 9.2%), vaccination complication (AV7909: 3.6%; BioThrax: 4.7%), musculoskeletal procedural complication (AV7909: 2.9%; BioThrax: 3.6%), 'procedural' or post-vaccination headache (AV7909: 2.8%; BioThrax: 4.9%), and injection site induration (AV7909: 2.2%; BioThrax: 2.9%).

There were no AE patterns or safety signals detected in Study EBS.AVA.212 for AV7909 (or BioThrax) vaccinated subjects, when assessed for up to 12 months after administration of the last dose of vaccine. All reported serious adverse events (SAEs) were unrelated to vaccination. Adverse events of special interest (AESIs) of potential autoimmune etiology were infrequent and were balanced between the AV7909 and BioThrax arms, 0.5% and 0.4%, respectively. Less than 0.1% of adjudicated AV7909 AESIs vs. 0.2% of adjudicated BioThrax AESIs were deemed vaccine related; with no discrete trend or pattern in AESIs observed. Reanalysis of safety data with revised datasets and exclusion of site US1027 did not alter safety findings or conclusions regarding safety endpoints assessed in EBS.AVA.212.

TEAEs that led to discontinuation of vaccination or study withdrawal were uncommon, as were deaths, other SAEs, and AESIs. In summary, AV7909 appeared to be generally well-tolerated with no significant safety concerns identified in EBS.AVA.212.

Summary of Study EBS.AVA.210: Human Interference Study of Ciprofloxacin and Doxycycline with AV7909

EBS.AVA.210 was a Phase 2, AV7909-antimicrobial interaction study in healthy adults 18-45 years of age, evaluating coadministration effects of AV7909 on antimicrobial PK and whether TNA levels two weeks following the final dose of a two-dose AV7909 vaccination series is affected by concomitant dosing with oral ciprofloxacin or doxycycline.

The primary PK endpoint for each antibiotic was considered met if the 90% confidence interval (CI) of the geometric mean of the within-subject ratios were contained entirely within the equivalence bounds of [0.8, 1.25] for both area under the curve from 0 to 12 hours (AUC_{0-12h}) and maximum concentration (C_{max}) at steady state. The secondary PK endpoint for each antibiotic was considered met if the 90% CIs of the geometric mean of the within-subject ratios were contained entirely within the equivalence bounds of [0.8, 1.25] for both AUC_{0-12h} and C_{max} after a single dose.

IM administration of a 2-dose regimen of AV7909 had no statistically significant effect on the steady-state of ciprofloxacin (primary PK endpoint), based on pre-specified PK equivalence criteria. For the secondary endpoints of single dose ciprofloxacin PK measurements AUC_{0-12h} and C_{max} , predefined equivalence criteria were not met, as they were slightly below the predefined equivalence criteria of [0.80, 1.25].

IM administration of a two-dose regimen of AV7909 resulted in 8-10% lower steady-state exposure of doxycycline. Based on pre-specified PK equivalence criteria for steady-state doxycycline AUC_{0-12h} and C_{max} , the primary PK endpoint for doxycycline was not met. For the secondary PK endpoint for single dose doxycycline, the first equivalence criterion was met (the 90% CI for the mean ratio for C_{max} [90% CI: 0.82, 1.24] was fully contained within the predefined equivalence criteria of [0.80, 1.25]), but the second equivalence criterion was not met (the upper

bound of the 90% CI of the mean ratio for AUC_{0-12h} [90% CI: 0.86, 1.28] was slightly above the predefined upper equivalence limit of 1.25).

No appreciable effect on AV7909 immunogenicity was observed with coadministration of antimicrobial therapy. Although PK results for single dose ciprofloxacin administration and for steady state and single dose doxycycline administration did not meet pre-specified success criteria, the nominal decrement in AUC_{0-12h} and C_{max} (approximately 5-7% and 8-10% lower systemic exposure for single-dose ciprofloxacin and steady state doxycycline, respectively) were not considered clinically significant.

Safety evaluation in EBS.AVA.210 indicated that AV7909 administered alone or in combination with ciprofloxacin or doxycycline throughout the entire study period was well tolerated and had an overall acceptable safety profile.

Due to differences in collection of reactogenicity data across studies, pooling of safety data from the four clinical studies was determined relevant only for increasing likelihood of detecting less commonly occurring events, such as SAEs and AESIs, and did not identify any new safety concerns pertaining to AV7909 administration. Study EBS.AVA.212 (n=3151 subjects who received AV7909) contributed the most subjects to the overall safety database, and the pooled safety data (n=3276 subjects who received the 'to-be-marketed' dosing regimen of AV7909) were consistent with results of that study.

Based on the submitted clinical data and in conjunction with nonclinical data, the clinical reviewer recommends approval under the Animal Rule of AV7909 administered IM at Week 0 and Week 2, for PEP against suspected or confirmed disease due to *B. anthracis* in adults 18-65 years of age, when given concomitantly with the recommended antibacterial regimen.

1.1 Demographic Information: Subgroup Demographics and Analysis Summary

EBS.AVA.212:

Subgroup analyses of immunogenicity (TNA NF_{50} on Days 29 and 64) and safety data (e-diary reactogenicity, TEAEs, and SAEs) in EBS.AVA.212 were tabulated by age group (18-30, 31-50, and 51-65 years), sex (male, female), and race (White, African American, Other/More than One Race). No formal statistical hypothesis testing was performed.

Immune responses trended higher in younger subjects (18-30 years). There was no significant difference in the immune response in AV7909 vaccinated subjects when evaluated by sex or racial subgroup. Subgroup analyses of immunogenicity did not change appreciably after data from site US1027 were excluded.

A slightly greater proportion of injection site reactions (local and systemic reactogenicity) and a slightly higher prevalence of more severe reactions (Grade 2 or 3) after AV7909 administration was observed in the youngest subgroup of subjects (18-30 years of age) and in female subjects. No trend in local or systemic reactogenicity (across all symptoms evaluated) was consistently observed related to AV7909 vaccination or dose number when assessed by racial subgroups.

For TEAEs, safety findings in the subgroup categories were consistent with the results in the overall Safety Population. Although the study was not powered to detect treatment between subgroups, the safety profile of AV7909 was generally consistent across age, sex, and racial subgroups. Similar trends were observed in the demographic subgroups excluding site US1027 data. There was no trend, pattern, or temporal association in SAEs reported with AV7909

administration; therefore, subgroup analysis of this safety endpoint was not useful for informing AV7909's safety profile across demographic subgroups.

EBS.AVA.210:

Immunogenicity assessments in EBS.AVA.210 by age and sex indicated no notable differences in geometric mean TNA NF₅₀ ratios and corresponding 95% CIs calculated using the primary (unadjusted) and exploratory (adjusted for site, sex, and age) analyses. Safety evaluation for demographic subgroups was not prospectively evaluated in EBS.AVA.210.

1.2 Patient Experience Data

No patient experience data were submitted by Applicant.

2. CLINICAL AND REGULATORY BACKGROUND

2.1 Disease or Health-Related Condition(s) Studied

Anthrax is a life-threatening acute infectious disease caused by the spore-forming bacterium *B. anthracis*. Naturally occurring anthrax is rare in humans; however, it has potential for use as a bioweapon. Thus, the United States (US) government has prioritized development of anthrax PEP as part of bioweapon emergency response measures.

Anthrax can occur in cutaneous, gastrointestinal, and inhalational forms, with inhalational anthrax having a particularly high mortality rate.¹⁰ Inhalational (pulmonary) anthrax has been reported to occur from 1-43 days after exposure to aerosolized spores. The fatality rate for inhalational anthrax in the US is approximately 45% to 90%. From 1900 to October 2001, there were 18 identified cases of inhalational anthrax in the US, the latest of which was reported in 1976, with an 89% (16/18) mortality rate. Most of these exposures occurred in industrial settings, such as textile mills. From October 4, 2001, to December 5, 2001, a total of 11 cases of inhalational anthrax linked to intentional dissemination of *B. anthracis* spores were identified in the US; 5 of these 11 cases were fatal.¹⁰

The spore form of *B. anthracis* is the predominant phase of the bacterium in the environment, and it is largely through the uptake of spores that anthrax disease is contracted.¹⁰ In humans, anthrax disease can result from contact with hides, leather, or hair products from contaminated animals or from other exposures to *B. anthracis* spores. Spore forms are markedly resistant to heat, cold, pH, desiccation, chemicals, and irradiation. Specifically, with inhalational anthrax, inhaled spores migrate to the lymph nodes, where they germinate. Following germination at the site of infection, bacilli can enter the blood and cause septicemia. The production of large quantities of anthrax toxin is believed to play a critical role in disease symptomatology and progression.

Anthrax toxin is composed of three proteins which confer virulence: PA, lethal factor (LF), and edema factor (EF). By itself, PA is non-toxic, but when combined with LF or EF, lethal toxin (LT) or edema toxin (ET), respectively, is formed. These toxins inhibit the innate immune response, allowing the bacteria to replicate unchecked in host cells. On a cellular level, this occurs through alteration of cellular signaling and metabolism. Due to the toxin's critical role in disease pathogenesis, neutralization of the toxin may attenuate or ameliorate disease. Since PA is part of both LT and ET, antibodies that inhibit the action of PA would be expected to neutralize both.

2.2 Currently Available, Pharmacologically Unrelated Treatment(s)/Intervention(s) for the Proposed Indication(s)

Antimicrobial therapy, Anthrax Vaccine Adsorbed (AVA, BioThrax), hyper immune globulin, and monoclonal antibodies that target anthrax proteins, and supportive critical care are the currently available interventions for PEP against anthrax.^{10,11}

BioThrax, (AVA, Emergent Product Development Gaithersburg, Inc.) is FDA-approved for PEP against anthrax in persons 18-65 years of age following suspected or confirmed *B. anthracis* exposure, when administered in conjunction with recommended antimicrobial drugs. It is administered SC as a three-dose series at Weeks 0, 2, and 4.

Four antibiotics are FDA-approved for use for PEP following exposure to aerosolized spores of *B. anthracis*: doxycycline, ciprofloxacin, levofloxacin, and parenteral procaine penicillin G.^{10,11} The Centers for Disease Control and Prevention (CDC) recommends that levofloxacin be reserved as a second-line agent, as safety data on its use in treatment for longer than 28 days are limited.¹²

Despite antibiotic treatment, anthrax can be fatal because antibiotics are not effective against non-germinating spores or toxins. In addition, anthrax spores can survive in the host for extended periods (as observed in NHPs up to 100 days post-exposure) and can germinate and release toxins after discontinuation of antibiotics.⁸ Because of spore latency, a 60-day course of antibiotics (ciprofloxacin or doxycycline) in conjunction with three IM doses of BioThrax administered two weeks apart (0, 2, and 4 weeks) is recommended by the Advisory Committee on Immunization Practices (ACIP) for PEP of anthrax.^{10,11}

Other important factors may limit the effectiveness of antibiotic therapy for PEP of anthrax; these include time to initiation of treatment, and duration of and adherence to the antibiotic regimen. Data from the 2001 anthrax letter attacks suggested that adherence to the prescribed antibiotic regimen was low. Only 44% of 6178 respondents reported taking the prescribed antibiotics for up to 60 days.¹⁰ Lastly, the threat of multi-drug resistant (MDR) anthrax, per the Secretary of the Department of Homeland Security's material threat determination (MTD), issued on September 22, 2006,^{13,14} highlights the need for careful consideration of alternative post-exposure treatment approaches against *B. anthracis* strains resistant to one or more antibiotics.¹⁵⁻¹⁷

Three immunoglobulin derived products are available in the US to treat anthrax disease. Monoclonal antibody products, such as raxibacumab¹⁸ and obiltoximab,¹⁹ are FDA approved for prophylaxis of inhalational anthrax when alternative therapies are not available, or not appropriate. Raxibacumab is an FDA-approved (2012) recombinant immunoglobulin G1 (IgG1) human monoclonal antibody targeting the PA component of the LT of *B. anthracis*, for the treatment of inhalational anthrax in combination with appropriate antibacterial drugs for adult and pediatric patients, and for prophylaxis of inhalational anthrax when alternative therapies are not available or are not appropriate.¹⁸ Anthim (obiltoximab, Elusys) is a monoclonal antibody that binds to the PA component of *B. anthracis* and neutralizes toxins produced by *B. anthracis*.¹⁹ It is approved to prevent inhalational anthrax when alternative therapies are not available or appropriate. Both Raxibacumab and Anthrasil were licensed by FDA using the Animal Rule. Anthrasil²⁰ is an FDA-approved polyclonal human immune globulin (derived from the plasma of individuals who have been vaccinated with BioThrax vaccine) indicated for the treatment of inhalational anthrax in combination with appropriate antibacterial drugs for adult and pediatric patients.

2.3 Safety and Efficacy of Pharmacologically Related Products

AVA drug product, which is used to formulate AV7909, contains the same ingredients as the licensed BioThrax vaccine. BioThrax is a licensed vaccine indicated for the active immunization for the prevention of disease caused by *B. anthracis* in persons 18-65 years of age. It is licensed for both pre-exposure and PEP of anthrax in adults, with different dosing schedules for each clinical indication.

Licensure of BioThrax for PEP against anthrax disease by the Animal Rule (21 CFR 601 Subpart H for Biologics) was supported by bridging human immunogenicity data to immunogenicity threshold data obtained from two relevant animal species (NHPs and rabbits) associated with a 70% probability survival when animals were exposed to a lethal dose of anthrax.

Clinical data in support of the safety and effectiveness of BioThrax for the PEP indication against anthrax in healthy adult subjects 18-65 years of age comprised two clinical studies, EBS.AVA.005 and EBS.AVA.006. The first of these two studies, EBS.AVA.005, was designed to determine the appropriate dosing schedule of BioThrax for the PEP indication and evaluate different immunogenicity endpoints that would be bridged to animal immunogenicity and survival data to determine a threshold of protection. Using information obtained from study EBS.AVA.005, both anti-PA antibody levels and TNA NF₅₀ levels were further evaluated in a larger, pivotal Phase 3 study where resultant human antibody levels were bridged to protective antibody levels derived from animal challenge studies (rabbit general use prophylaxis [GUP] Study 646 and NHP Study 844) to support licensure under the Animal Rule.

The primary immunogenicity endpoint in EBS.AVA.006, defined as the percentage of subjects achieving a TNA response of at least 0.56 at Day 63 and correlated to a 70% survival rate of rabbits against oral inhalational anthrax challenge of 50% lethal dose by 200-fold (200 LD₅₀), met the pre-specified success criteria. Pre-defined secondary endpoints were also met.

Extensive safety data are available for BioThrax since its approval on November 4, 1970 (US) and are summarized in Dr. Jane Woo's (CBER, Division of Pharmacovigilance [DPV]) review memorandum. The most common (>10%) local (injection-site) adverse reactions observed in clinical studies were tenderness, pain, erythema, edema, and arm motion limitation (AML). The most common (≥5%) systemic adverse reactions were muscle aches, fatigue, and headache.³

A Phase 3, open-label, uncontrolled, multi-center study evaluated the three-dose PEP BioThrax schedule (Week 0, 2, and 4) in 200 healthy adult subjects.^{3,4,5} The most common solicited adverse reactions reported 7 days after each vaccination comprised local reactions, including symptoms of lump, tenderness, and erythema. The most common solicited systemic reactions comprised fatigue, headache, and myalgia. Of the subjects that reported local and systemic solicited reactions, ≥ 98% required minimal or no treatment and resulted in little to no interference with subjects' daily activity. The most common (> 2.0%) unsolicited related adverse reactions reported following at least one dose up to 100 days after the third dose were: headache (4.0%), fatigue (3.5%), skin hyperpigmentation (3.5%), decreased joint range of motion (2.5%), and myalgia (2.5%). No deaths were reported and neither of the two SAEs reported were considered related to vaccination.

2.4 Previous Human Experience with the Product (Including Foreign Experience)

Currently, AV7909 is not licensed in any country. Aside from the clinical studies conducted by the Applicant to support this BLA, a Biomedical Advanced Research and Development Authority

(BARDA)-sponsored Phase 2 clinical study evaluated AV7909 in an elderly population^{21,22} but was not submitted as part of the BLA submission.

Reviewer comment: *Results of this published study were not reviewed by FDA. BARDA did not provide datasets to FDA to verify the data and study data were not included by the Applicant (Emergent) in their BLA submission.*

CpG 7909 has been studied extensively in clinical trials, but to date, remains unlicensed in the U.S. as an adjuvant or monotherapy. Many of these studies were conducted for various cancer indications where CpG 7909 was administered by different routes (SC, intradermal [ID], intravenous [IV], intracerebrally, and intratumorally).^{23, 24} Results of these CpG studies have been difficult to interpret due to the heterogeneous population of cancer patients (n >2000) receiving various vaccines and antigenic tumor peptides, some with chemotherapy and other immunomodulators.

CpG 7909 has also been studied or is currently being studied as an adjuvant for various infectious disease indications (hepatitis B, malaria, influenza, and human immunodeficiency virus [HIV], and COVID-19).^{23, 25-27} Available information about the clinical trial data suggests that CpG 7909 has been generally well tolerated at the doses administered (e.g., up to doses of 1.05 mg/kg for the IV route, 10-40 mg for the SC route).

2.5 Summary of Pre- and Post-submission Regulatory Activity Related to the Submission

- **March 1, 2010:** Type B, Pre-Investigational New Drug (IND) Meeting Submission for AV7909
- **September 10, 2010:** Emergent submitted an IND application (IND #14451) for AV7909
- **November 16, 2010:** VRBPAC meeting² to consider the pathway to licensure for PA-based anthrax vaccines for a PEP indication using the Animal Rule.
 - During this meeting, agreement was reached on the selection of the appropriate animal efficacy models to bridge protection from animals to humans according to the following:
 - Pre-exposure animal model studies (rabbit and NHP) to serve as pivotal efficacy studies to estimate protective antibody levels in animals and extrapolate protection in animals to humans via an antibody bridge,
 - Post-exposure animal model studies (rabbit and NHP) to serve as proof-of-concept studies indicating that the vaccine provides added protection compared to antimicrobial treatment alone when administered concomitantly with antimicrobials in a post-exposure setting (supportive studies), and
 - Passive immunization animal studies (rabbit) to serve as proof-of-concept studies demonstrating that antibodies generated by humans vaccinated with BioThrax (and by extension, AV7909) could provide protection against exposure and, therefore, constitute an appropriate correlate of protection (supportive studies).
- **June 6, 2011:** FDA granted Emergent's request for Fast Track Designation
- **October 15, 2012:** End-of-Phase 1 Meeting
- **August 31, 2015:** End-of-Phase 2 Meeting
- **March 17, 2016:** Full Waiver for Pediatric Studies (agreed iPSP)
- **December 21, 2018:** Pre-Emergency Use Authorization (EUA) (CDC) for Distribution to US Strategic National Stockpile (SNS) (IND # 18715)
- **April 27, 2021:** Pre-BLA Meeting, Chemistry, Manufacturing, and Controls (CMC) (Written Responses Only [WRO])
- **October 12, 2021:** Pre-BLA Meeting, Clinical and Non-Clinical (WRO)
- **August 19, 2021:** Orphan Drug Designation (DRU#-2021-8325)

- **December 14, 2021; April 20, 2022:** Parts 1 and 2, respectively, of Rolling BLA STN 125761/0 submitted to FDA
- **June 17, 2022:** BLA filed with Standard Review (see Section 5.2 below for a summary of the basis for denial of the Priority Review Designation for AV7909 for anthrax PEP)

Reviewer comment: *The Applicant employed the approach endorsed at the November 16, 2010, VRBPAC workshop² in which animal efficacy studies and bridging to human clinical immunogenicity data (TNA titers) were used to support this application for licensure of AV7909 for PEP under the Animal Rule.*

2.6 Other Relevant Background Information

No other relevant background information was identified.

3. SUBMISSION QUALITY AND GOOD CLINICAL PRACTICES

3.1 Submission Quality and Completeness

The submission was adequately organized and integrated to accommodate the conduct of a complete clinical review without unreasonable difficulty. The Applicant submitted standardized Study Data Tabulation Model (SDTM) datasets and Analysis Data Model (ADaM) datasets for the two key studies, EBS.AVA.210 and -212.

3.2 Compliance with Good Clinical Practices and Submission Integrity

The FDA Office of Compliance and Biologics Quality (OCBQ), through its Bioresearch Monitoring Program (BIMO), conducted inspection of six clinical sites (sites US1003, US1006, US1008, US1019, US1026) for the pivotal clinical study (EBS.AVA.212) and (US2002) for EBS.AVA.210. A for-cause inspection was conducted for site US1027 (see reason for this inspection of US1027, below).

No significant deviations were observed for sites US1006, US1008, US1026 (minor deviation identified, site issued an NAI Letter), and US2002. Several deviations were reported for Sites US1003 (enrollment and dosing of an ineligible subject, unreported AEs, and concomitant medications for 3 subjects, inadequate follow-up and evaluation of AEs) and US1019 (adequate case histories for screening visits for 27 subjects, inaccurate source documentation for 3 subjects). Both of these sites were issued FDA Form 483s. FDA determined that the principal investigator (PI)'s corrective actions for these two sites were adequate.

Good clinical practice (GCP) issues were identified for Study EBS.AVA.212. Data integrity issues identified at this clinical site US1027 included data alteration to allow for inclusion of study ineligible subjects, failure to document AEs, and concomitant use of medications, including those that would qualify as protocol deviations.

After the original clinical study report (CSR) for EBS.AVA.212 was completed on May 6, 2021, the Applicant was informed on September 8, 2021, by their study contract research organization (CRO), (b) (4), of GCP issues at site US1027 (Iowa Clinic). (b) (4) report included allegations of the following:

1. Data alteration at the Screening visit for three subjects (US(b) (6) , US(b) (6) and US(b) (6) that included changes to body weight and height measurements that impacted body mass index (BMI) calculation so that study ineligible subjects would meet

inclusion criteria pertaining to BMI requirements (Inclusion Criterion #4: BMI \leq 35.0 kg/m² at Screening visit), and

2. Revision of treatment periods for prohibited medications to allow enrollment of subjects who would have been excluded based on inadequate time intervals between prohibited medication use and study enrollment (e.g., for Subject US(b) (6) , a change of start year for an antifungal medication (Lamisil) was made to avoid exclusion criterion #3 (Chronic administration [...] of immunosuppressants or other immune modifying drugs [...] within six months prior to the vaccine dose), although Lamisil would not have been an exclusionary prior medication per protocol (PP).

The Applicant was not able to procure (b) (4) audit report for site US1027 and conducted an independent investigation through the CRO (b) (4) , to confirm the data integrity issues reported by (b) (4) , and to assess if these issues extended beyond the initial report of the three impacted subjects, and whether they were isolated to site US1027 or if similar issues occurred at other study sites for EBS.AVA.212.

Reviewer comment: FDA requested that the Applicant provide the complete study audit report from (b) (4) for EBS.AVA.212 for review (Information Request [IR] #10), to better understand the type and extent of the issues identified at study site US1027, to validate the decision to exclude immunogenicity and/or safety data from study site US1027 from clinical data analyses for Study 212 and determine if additional analyses would be needed. The Applicant provided (b) (4) independent study audit investigation of site US1027 in response to FDA's request under STN 125761/0/17, submitted on September 19, 2022.

(b) (4) study audit report (b) (4) 041971) showed that data integrity issues extended beyond the initial report of data alterations for the three subjects at site US1027 but were limited to site US1027 and not found at any other study sites.

(b) (4) (major) audit findings for EBS.AVA.212 are summarized, as follows:

- A significant lack of data integrity observed for a substantial number of vital sign (VS) measurements collected at Screening (e.g., height and weight) and used to calculate the BMI needed for evaluating subject eligibility for study enrollment (e.g., incorrect conversions of height from feet to inches that affected BMI calculation for some enrolled subjects).
- Six (6) of 17 (35%) selected subjects sampled for audit review of the source electronic medical records (EMR) data were found to have one or more AEs that had not been included in the hardcopy subject record file, noted on the AE log, or otherwise reported for consideration and analysis. As stated in the audit report: "Source records were observed to contain information identifying AEs, although the AE log stated, "no AEs reported" (or similar)." This error appeared to stem from an apparent failure to include data from the site EMR in the study data (i.e., in the hardcopy subject files).
- New concomitant medications prescribed/taken during the study were not identified and documented within the log of concomitant records in each subject's case file. A few of the unreported medications may potentially have been protocol deviations.
- Source records of two "critical" eligibility protocol deviations were found to be inadequately managed.
- Good Documentation Practices were often not followed.

Based on the GCP issues identified by (b) (4) audit, site US1027 was further investigated by FDA's BIMO to confirm and assess the reported GCP findings. The FDA BIMO investigator's

assessment of site US1027 confirmed some, but not all, of the Applicant's audit findings (site US1027 was issued an FDA form 483, Inspectional Observations, for these violations). The FDA BIMO investigator observed that the site's PI and sub-investigators personally reviewed the eligibility of each subject, signed each informed consent form (ICF), reviewed, and signed off on all safety assessments and safety reports in a timely manner, reviewed all identified protocol deviations, met frequently with study staff members, and took immediate action when the issues were identified.

Reviewer comment: Based on (b) (4) audit findings and FDA BIMO investigation findings, FDA determined that data from site US1027 should be excluded from the immunogenicity and safety analyses for Study 212.

Based on (b) (4) and (b) (4) s audit findings identified for site US1027 in Study EBS.AVA.212, the Applicant preemptively updated the immunogenicity and safety analyses of EBS.AVA.212 to exclude data from site US1027 under STN 125761/0, EBS.AVA.212 CSR Addendum 2 (submitted in Part 2 of the BLA on 20 April 2022).

The revised datasets resulted in changes to the safety summaries for studies EBS.AVA.210 and -212 and the Integrated Summary of Safety (ISS). Due to the extensive changes made during review, the amendment containing the majority of these revisions (STN 125761/0/15) was designated a Major Amendment (MA).

Reviewer comment: The immunogenicity results (PP Population) presented in CSR Addendum 2 (without site US1027 immunogenicity data) represent the overall study immunogenicity conclusions. The Applicant included safety data from site US1027 in the proposed United States Prescribing Information (USPI), citing two reasons for this inclusion:

- Subjects who received the study vaccinations (including AV7909) at site US1027 reported solicited (local and systemic) reactogenicities and unsolicited AEs; and
- The Applicant believed that there was no evidence that the integrity of the safety information at site US1027 was affected by the GCP issues identified at the site.

The clinical reviewer disagreed with the Applicant's rationale for inclusion of data from site US1027 in the safety data analysis. FDA's review of the independent study audit report from (b) (4) indicated that the integrity of safety data was compromised by GCP issues (e.g., not reporting AEs), therefore the clinical reviewer did not consider inclusion of study site US1027 data appropriate in the safety analysis for Study EBS.AVA.212 (See Sections 5.2 and 6.1.9). Inclusion of subjects from site US1027 in the denominator when calculating rates of safety events, when the collection was insufficient to ensure they were captured in the numerator, would dilute the AE rates reported. All safety data presented in the clinical review of EBS.AVA.212 and the ISS exclude safety data from site US1027.

Apart from data integrity issues identified by (b) (4), the FDA statistical reviewer identified several additional issues which compromised the integrity of safety data submitted for studies EBS.AVA.210 and -212. These included:

- Data discrepancies observed between the revised study datasets for EBS.AVA.210 and -212 submitted in STN 125761/0/15 and the source tables presented in the EBS.AVA.210 and -212 CSR addenda, submitted in STN 125761/0/27 (IR #19).
- Inclusion of 'missing' subjects (who did not provide any e-diary data) in the denominator of subjects for the Safety Population for the calculation of the percentage of subjects in

the Safety Population who reported solicited reactions for studies EBS.AVA.201, -208, -210, and -212 (IR #19).

- Reporting of solicited reactions attributed to the incorrect dose number (VACCNUM; ADFACE dataset) in five subjects in EBS.AVA.212 (IR #23), and
- Reporting of AEs in six subjects (EBS.AVA.210 and -212) as TEAEs which were AEs that occurred prior to administration of the first vaccine dose or antibiotic (IR #23).

Reviewer comment: *In response to the statistical reviewer's findings, FDA requested submission of corrected ADFACE and ADAE datasets and tables to address these discrepancies, where applicable.*

Unless specified otherwise, 'revised' datasets (for Studies EBS.AVA.210, -212, and the ISS) refer to revised AE and CE (SDTM and AdAM) datasets that exclude site US1027 for Study EBS.AVA.212; and incorporate revisions to Studies EBS.AVA.210, -212 and the ISS that correct the data discrepancies reported by the statistical reviewer in IRs #19 and #23 (outlined above). Clinical datasets submitted under STN 125761/0/27 and as incorporated in CSR Addendum 3 for Studies EBS.AVA.210, -212, and the ISS include these revised datasets.

3.3 Financial Disclosures

The Applicant has adequately disclosed financial interests/arrangements with clinical investigators as recommended in the Guidance for Industry Financial Disclosure by Clinical Investigator.

No conflicts of interest were reported for any of the clinical investigators (PIs) involved in Studies EBS.AVA.201, -208, -210, or -212.

There were no conflicts of interest identified for any sub-investigators in the clinical studies with one exception. Financial disclosure information was received from Celia Gonzalez, a sub-investigator (Sub-I) participating in Study EBS.AVA.212 at site US1007 (PI: Carlos Fierro, MD). The spouse of this Sub-I worked as a full-time employee of the managing (b) (4) [REDACTED], during the conduct of the study (Salary: USD (b) (6) [REDACTED]).

Reviewer comment: *The reported potential conflict of interest of the spouse of one of the sub-investigators in Study 212 having worked for the managing CRO (cited above) is unlikely to have impacted study results for Study 212.*

4. SIGNIFICANT EFFICACY/SAFETY ISSUES RELATED TO OTHER REVIEW DISCIPLINES

4.1 Chemistry, Manufacturing, and Controls

AV7909 consists of AVA bulk drug substance and CpG 7909 (a synthetic oligonucleotide) adjuvant. The AVA drug substance is similar in composition and manufacturing process to BioThrax and shares the major antigenic component (PA) with BioThrax. CpG 7909 is an immunostimulatory synthetic ODN that functions as an adjuvant by activation of TLR9. CpG 7909 is designed to induce both an enhanced antigen-specific antibody response and a natural killer T-cell response when used in combination with prophylactic or therapeutic vaccines.^{23,28,29}

Please refer to the CMC and adjuvant reviewers' respective reviews for a comprehensive assessment of the drug substance (AVA), drug product (AVA Adsorbed with aluminum

hydroxide with CpG 7909 adjuvant) and CpG 7909 adjuvant for further details pertaining to manufacture of these components of AV7909.

4.2 Assay Validation

No substantive issues were identified during the review of the toxin neutralizing antibody (TNA) assay. This methodology was previously reviewed under STN 103821/5344. Please see the CMC review memorandum for details (Anita Verma., Ph.D., CBER, OVR, DBPAP).

4.3 Nonclinical Pharmacology/Toxicology

No pharmacology/toxicology issues were identified that would preclude approval of this BLA. Please refer to the pharmacology/toxicology reviewer's (Dr. Claudia Wrzesinski) review for a complete discussion of the pharmacology/toxicology studies conducted to support licensure of AV7909 for PEP against disease due to anthrax exposure.

4.4 Clinical Pharmacology

4.4.1 Mechanism of Action

AVA is prepared from a sterile filtrate culture fluid of an avirulent, nonencapsulated strain of *B. anthracis* and contains proteins, including the 83kDa PA protein released during the growth period and contains no dead or live bacteria. Although an immune correlate of protection is unknown, antibodies raised against PA may contribute to protection by neutralizing the activities of anthrax toxins. *B. anthracis* proteins other than PA may be present in AVA, but their contribution to protection has not been determined.

CpG 7909 is a synthetic immunostimulatory ODN that is a TLR9 agonist designed to induce an enhanced antigen-specific antibody response and natural killer T-cell immune response when used in combination with preventive or therapeutic vaccines. It stimulates TLR9-expressing cells (including human plasmacytoid dendritic cells and B cells) to induce an innate immune response characterized by the production of T-helper type 1 (Th1) cells and proinflammatory cytokines.²⁸ CpG 7909 adjuvant is a novel formulation not approved in any pharmaceutical product by the FDA.

4.4.2 Human Pharmacodynamics (PD)

Human pharmacodynamic studies to assess the effectiveness of AV7909 for the prevention of anthrax disease after exposure to *B. anthracis* are not ethically permissible, due to the lethal nature of anthrax toxin. Field studies are not feasible because naturally occurring anthrax in humans is extremely rare.⁹ Therefore, effectiveness of AV7909 in a post-exposure setting was evaluated in two animal species (guinea pigs and NHPs) determined to be appropriate animal models based on fulfillment of the four criteria of the Animal Rule (21 CFR 601 Subpart H):

- that there is a reasonably well-understood pathophysiological mechanism of the toxicity of the agent and its prevention or substantial reduction by the product,
- the effect is demonstrated in more than one animal species expected to react with a response predictive for humans,
- the animal study endpoint is clearly related to the desired benefit in humans, which is generally the enhancement of survival or prevention of major morbidity, and
- the data or information on the kinetics and pharmacodynamics (PD) of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans.

A scientific strategy for bridging animal protection data to humans for PA-based vaccines, including BioThrax, was agreed upon at the November 16, 2010, VRPBAC workshop² and was based on data obtained from a combination of three different study designs: a GUP study, a PEP study, and a passive immunization study. For additional details pertaining to the animal studies used to support licensure of AV7909 for anthrax PEP, please refer to the non-clinical reviewer's (Dr. Tod Merkel; DBPAP) memorandum.

4.4.3 Human Pharmacokinetics (PK)

One clinical PK study was submitted to this BLA, Study EBS.AVA.210, and is presented in detail in Section 6.2 of this clinical review.

4.5 Statistical

No statistical issues were identified by the statistical reviewer, Dr. Ye Yang, that would preclude approval of this BLA submission. Please see the statistical review for details.

4.6 Pharmacovigilance

The Applicant provided a synopsis of their proposed postmarketing field study, Study EBS.AVA.213, entitled "A Phase 4, Retrospective, Observational Study of AV7909 Anthrax Vaccine for Post-Exposure Prophylaxis Following a *Bacillus Anthracis* Mass Exposure Event" in their pharmacovigilance plan (PVP), as required under 21 CFR 601.91(b)(1). EBS.AVA.213 will be completed upon case review of up to 250 confirmed cases of inhalational anthrax and/or anthrax meningitis or after a given outbreak results in up to 10,000 individuals having received AV7909 vaccine with concurrent recommended antibacterial drugs. Key clinical benefit endpoints comprise a determination of the incidence rate of anthrax disease and survival outcomes in subjects given the PEP schedule of AV7909, concurrently with 60 days of antimicrobials. Key safety endpoints comprise a description of all AEs and SAEs associated with the AV7909 PEP schedule during an anthrax exposure incident. The pharmacovigilance reviewer did not identify any significant deficiencies in the postmarketing study synopsis submitted to the BLA. A separate pregnancy registry for this product will not be conducted; as maternal fetal outcomes will be assessed as part of the safety evaluation in EBS.AVA.213. The PVP reviewer determined that the available safety data do not substantiate a need for a Risk Evaluation and Mitigation Strategy (REMS). Please refer to the reviewer's (Dr. Jane Woo, DPV, Office of Biostatistics and Pharmacovigilance [OBPV]) evaluation of the proposed Phase 4 field study of AV7909 in the event of a mass anthrax event.

Reviewer comment: *The proposed postmarketing requirement (PMR) field study is similar in design to that proposed for BioThrax for the PEP indication (STN 103821/5344). The study's design satisfactorily addresses CBER recommendations communicated to the Applicant in AV7909 pre-BLA meeting comments dated October 12, 2021.*

5. SOURCES OF CLINICAL DATA AND OTHER INFORMATION CONSIDERED IN THE REVIEW

5.1 Review Strategy

The clinical reviewer focused on the two pivotal clinical studies, EBS.AVA.210 and -212, with targeted review of the Phase 1 study EBS.AVA.201 and Phase 2 study EBS.AVA.208. An Integrated Summary of Efficacy (ISE) was not provided in this submission, as per FDA's advice communicated to the Applicant in pre-BLA meeting comments dated October 12, 2021. The ISS

section of the BLA was reviewed and summarized. The clinical reviewer also reviewed the proposed field study for AV7909 in Section 4.6., Pharmacovigilance, discussed above.

5.2 BLA/IND Documents That Serve as the Basis for the Clinical Review

BLA 125761/0 was submitted as a rolling submission in two parts. The first part of the BLA was submitted electronically on December 14, 2021 (CMC, Pharmacodynamics and Pharmacology/Toxicology section). The clinical portion of STN 125761/0 was submitted electronically as the second and final part of the rolling BLA submission on April 20, 2022. Included in the second part of STN 125761/0 was the Applicant's request for Priority Review Designation, which was reviewed by FDA and denied (see discussion below for FDA's rationale for denial of PR).

Documents in STN 125761/0 reviewed by the clinical reviewer include the following:

- Module 1: M1.2, 1.3, 1.4, 1.6, 1.7, 1.9., 1.11, 1.12, 1.13, 1.14, 1.16, and 1.17
- Module 2: M2.2, 2.4, 2.5, 2.6, 2.7
- Module 5: M5.2, 5.3, 5.3.5, 5.3.5.1, 5.3.5.2, 5.3.5.3, 5.3.5.4, and 5.4

Priority Review (PR) Designation Request was reviewed under Module 1.2.

FDA denied the Applicant's PR request, due to insufficient data to meet the second requirement for PR designation that "if approved, the drug or biologic would provide a significant improvement in the 'safety or effectiveness of the treatment, prevention, or diagnosis of a serious condition' over available therapy", based on the following rationale:

- AV7909 achieving a peak TNA NF₅₀ response at an earlier timepoint following fewer doses does not constitute a "significant improvement in effectiveness" since its role in anthrax PEP is as an adjunctive therapy to be given in concert with recommended antibiotics.
 - FDA acknowledged that in Study EBS.AVA.212 the proportion of subjects achieving the designated TNA NF₅₀ threshold following AV7909 was higher than the proportion achieving this threshold following BioThrax, though the proportion for the BioThrax arm in this study was lower than the proportion reported in study EBS.AVA.006, as labeled in the BioThrax USPI; the clinical significance of this difference is uncertain.
 - FDA also acknowledged that the peak TNA NF₅₀ GMT was higher following AV7909 compared to BioThrax, but the clinical significance of this higher GMT is also uncertain. Therefore, FDA was not able to make a determination that AV7909 would provide a significant improvement over available therapy.

Additional materials which were used in the review of this BLA comprised responses (listed below) to numerous clinical IRs.

- STN 125761/0/3 (submitted on 4/20/22)
- STN 125761/0/4 (submitted on 6/16/22)
- STN 125761/0/6 (submitted on 7/19/22)
- STN 125761/0/7 (submitted on 7/20/22)
- STN 125761/0/15 (submitted on 9/9/22)
- STN 125761/0/17 (submitted on 9/19/22)
- STN 125761/0/19 (submitted on 9/23/22)
- STN 125761/0/20 (submitted on 9/28/22)
- STN 125761/0/22 (submitted on 10/5/22)

- STN 125761/0/27 (submitted on 11/18/22)
- STN 125761/0/31 (submitted on 12/20/22)
- STN 125761/0/37 (submitted on 02/24/23)
- STN 125761/0/38 (submitted on 2/28/23)
- STN 125761/0/42 (submitted on 4/8/23)
- STN 125761/0/51 (submitted on 06/08/23)
- STN 125761/0/52 (submitted on 06/12/23)
- STN 125761/0/53 (submitted on 06/20/23)

Reviewer comment: *The Applicant satisfactorily addressed all clinical IRs in the amendments listed above.*

5.3 Table of Studies/Clinical Trials

The clinical studies submitted to this BLA are summarized in Table 1:

Table 1. Summary of Clinical Studies for BLA 125761/0 Submitted in Support of Licensure of AV7909 for Anthrax Post-exposure Prophylaxis (PEP)¹

Study Number	Study Design	Dosing Regimen	Subject Populations (n)	Primary Endpoints
EBS.AVA.212² NCT# 03877926 Phase 3, Safety, Immunogenicity and Lot-to-lot Consistency Study in Healthy Adults 18-65 yrs of age.	Double-blind, randomized, multi-center, parallel arm	3 injections at 0, 2 and 4 weeks ³ Arm 1: AV909 Lot 1 Arm 2: AV909 Lot 2 Arm 3: AV909 Lot 3 Arm 4: BioThrax SC (AVA 0.5 mL)	<u>AV7909</u> : ITT: 3156 Safety: 3151 PP: 2543	Primary (Immunogenicity): <ul style="list-style-type: none"> Lot consistency: <ol style="list-style-type: none"> 1. GMT Ratio of TNA NF₅₀ at Day 64. 2. TNA NF₅₀ ≥0.56 at Day 64. Effectiveness: <ol style="list-style-type: none"> 1. AV7909 Immunogenicity at Day 64. 2. Comparison of the Percentage of Subjects with a TNA NF₅₀ ≥0.29. Primary (Safety): <ul style="list-style-type: none"> Evaluate the safety of AV7909 in healthy adults. Following a 2-dose AV7909 schedule administered IM.
EBS.AVA.210 NCT# 04067011 Phase 2, Vaccine-Drug Interaction Study in Healthy Adults 18-45 years of age.	Open label, randomized (1:1:2), multicenter, PK interaction study of AV7909 with ciprofloxacin and doxycycline	2 IM injections at 0 and 2 weeks Arm 1: AV7909 + ciprofloxacin Arm 2: AV7909 + doxycycline Arm 3: AV7909 alone	<u>AV7909</u> : ITT: 210 Safety: 190 Immunogenicity (PP): 151	Co-Primary (PK): <ul style="list-style-type: none"> AUC_{0-12h} and C_{max} for ciprofloxacin on Days 8 and 35. AUC_{0-12h} and C_{max} for doxycycline on Days 8 and 38.

Study Number	Study Design	Dosing Regimen	Subject Populations (n)	Primary Endpoints
EBS.AVA.201 NCT# 01263691 Phase 1 Immunogenicity and Safety in Healthy Adults 18-50 years of age.	Randomized (6:6:6:6:6:5), double-blind, placebo- controlled, dose ranging, parallel arms	2 IM injections 2 weeks apart (Day 0, 14): Arm 1: BioThrax (0.5 mL) Arm 2: 0.5 mL AVA + 0.5 mg CpG 7909 Arm 3: 0.5 mL AVA + 0.25 mg CpG 7909 Arm 4: 0.25 mL AVA + 0.5 mg CpG 7909 Arm 5: 0.25 mL AVA + 0.25 mg CpG 7909 Arm 6: Saline	ITT: 105 Safety:105 PP: 100	Primary (Safety): <ul style="list-style-type: none"> • Safety and tolerability, as determined by all AE incidence rates, of 4 AVA plus CpG 7909 (AV7909) formulations, compared to saline placebo and AVA alone. • Evaluation of local and systemic vaccine reactogenicity occurring within 7 days of dosing.
EBS.AVA.208 NCT# 01770743 Phase 2, Immunogenicity and Safety in Healthy Adults 18-50 years of age.	Randomized (4:3:2:4:2), multicenter, double-blind, active-controlled parallel arms	3 IM injections at 0, 2, 4 weeks: Arm 1: AV7909 ¹ , AV7909, saline Arm 2: AV7909 ¹ , saline, AV7909 Arm 3: AV7909 ¹ , AV7909, AV7909 Arm 4: ½ dose AV7909, ½ dose AV7909, ½ dose AV7909 Arm 5: BioThrax, BioThrax, BioThrax	ITT: 168 Safety: 168 Immunogenicity (PP): 144	Primary (Immunogenicity): <ul style="list-style-type: none"> • Percentage of Subjects with TNA NF₅₀ ≥ 0.56 at Day 63.

SC: Subcutaneous; IM: Intramuscular ITT: Intent-to-treat population; PP: Per Protocol Population; TNA NF₅₀: Toxin Neutralizing Antibody 50% neutralization factor.

NCT #: National Clinical Studies Database (clinicaltrials.gov) identifier number.

¹Reviewer generated table based on STN 125761/0, Section 2.7.3 Summary of Clinical Efficacy, Table 1, pages, 9-11; Section 2.7.6., Synopses of Clinical Studies, pages 1-88; and Section 5.2, Tabular Listing of All Clinical Studies, pages 1-2.

²Excludes site US1027 (see population numbers).

³AV7909 given by IM route.

5.4 Consultations

5.4.1 Advisory Committee Meeting (if applicable)

The Applicant employed the approach endorsed at the November 16, 2010, VRBPAC workshop² in which animal efficacy studies and bridging to human clinical immunogenicity data (TNA titers) were used to support this application for licensure of AV7909 for PEP under the Animal Rule. Thus, this BLA was not presented at VRBPAC.

5.4.2 External Consults/Collaborations

Consult Request to the Center for Drug Evaluation and Research (CDER), OND, DAI DIDP's for PK assessment of the AV7909-antimicrobial interaction Study EBS.AVA.210.

DVRPA requested a PK consultation from CDER (OND, DAI) on July 26, 2022 (ICCR# 00861829), regarding interpretation of the antimicrobial PK data of Study EBS.AVA.210. DAI's assessment was that although all the PK endpoints were not met (see Section 6.2), PK findings in EBS.AVA.210 did not indicate a clinically significant impact of AV7909 coadministration on the PK of ciprofloxacin or doxycycline. DAI's recommendations regarding revision of USPI Section 14.2 is provided in Section 11.5 of the clinical review memorandum.

5.5 Literature Reviewed

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6. DISCUSSION OF INDIVIDUAL STUDIES/CLINICAL TRIALS

6.1 Trial #1

A Phase 3, Randomized, Double-blind, Parallel-group Trial to Evaluate the Lot Consistency, Immunogenicity, and Safety of AV7909 for Postexposure Prophylaxis of Anthrax in Healthy Adults

6.1.1 Objectives

Primary Objectives:

- To demonstrate lot consistency following a two-dose schedule of AV7909 (Days 1 and 15) administered IM in healthy adults.
- To demonstrate immunogenicity under the FDA’s Animal Rule on Day 64 following a two-dose schedule of AV7909 (Days 1 and 15) administered IM in healthy adults.
- To demonstrate immunogenicity using the FDA’s Animal Rule on Day 64 based on the non-inferiority of a two-dose schedule of IM administered AV7909 (Days 1 and 15) to the licensed three-dose schedule of BioThrax (Days 1, 15, and 29) administered SC in healthy adults.
- To evaluate the safety of AV7909 in healthy adults following a two-dose schedule (Days 1 and 15) administered IM.

Secondary Objective:

- To demonstrate immunogenicity under the FDA’s Animal Rule on Day 29 following a two-dose schedule of AV7909 (Days 1 and 15) administered IM in healthy adults.

6.1.2 Design Overview

EBS.AVA.212 was a Phase 3, multicenter, randomized, double-blind, parallel-group trial designed to evaluate the lot consistency, immunogenicity, and safety of a 2-dose schedule of AV7909 (b) (4) AVA plus (b) (4) mg CpG 7909 adjuvant) administered IM on Days 1 and 15, in healthy adults (18-65 years of age), for PEP against anthrax disease. BioThrax (AVA, Emergent Product Development Gaithersburg, Inc.) given per the PEP schedule as 3 SC injections on Days 1, 15, and 29, was the active comparator for this trial. Saline placebo was given SC at Day 29 in the AV7909 groups.

Subjects meeting study eligibility criteria at screening 2 to 28 days prior to randomization were randomized 2:2:2:1 (block size of seven) via an interactive voice and/or web response system (IxRS) to one of the four study groups on Day 1, as shown in Table 2, below. Randomization was stratified by site. Randomized subjects who withdrew from the study for any reason were not replaced.

Table 2. Study EBS.AVA.212: Study Groups

IP Group	Treatment	Sample Size	Day 1	Day 15	Day 29
1	AV7909 Lot 1	1100	AV7909 Lot 1 (IM)	AV7909 Lot 1 (IM)	Placebo (SC)
2	AV7909 Lot 2	1100	AV7909 Lot 2 (IM)	AV7909 Lot 2 (IM)	Placebo (SC)
3	AV7909 Lot 3	1100	AV7909 Lot 3 (IM)	AV7909 Lot 3 (IM)	Placebo (SC)
4	BioThrax	550	BioThrax (SC)	BioThrax (SC)	BioThrax (SC)
Total	--	3850	--	--	--

IP: Investigational Product IM: Intramuscular SC: Subcutaneous
Ref: BLA STN 125761/0, EBS.AVA.212, CSR, Table 2, page 21 of 247.

Double blinding was ensured by: (1) administering injections in an area of the clinical site apart from blinded site personnel, (2) masking the syringe barrel to obscure the contents and (3) by instructing the subject to look away from the syringe during vaccination preparation and as the injection was administered. Only the site pharmacists or other designated study personnel who prepared and/or administered the vaccinations were unblinded to investigational product (IP) assignment.

Reviewer comment: *The study's randomization and blinding procedures were appropriate.*

Individual participation in this study was approximately 15 months. Four in-clinic visits occurred over 2 months (i.e., Days 0, 15, 29, and 64). Safety follow-up phone calls were conducted at Day 43 and Month 4 (Day 120), Month 7 (Day 211), Month 10 (Day 302), and Month 13 (Day 394), i.e., nominally 0.5, 3, 6, 9, and 12 months after the last vaccination, to collect information on TEAEs, SAEs, and AESIs.

6.1.3 Population

Key Eligibility Criteria

Inclusion Criteria:

- Male or female subjects 18-65 years of age, inclusive, at the time of informed consent.
- BMI ≤ 35.0 kg/m² at the Screening visit.
- Women of childbearing potential (WOCBP) not pregnant and on appropriate contraception.

Key Exclusion Criteria:

- Use of any IP (drug, vaccine, device, or combination product) within 30 days preceding the first dose of study vaccine, or planned use during the study through Month 13.
- Planned administration of any commercially available vaccine from seven days prior to the first study vaccination through two weeks after the last vaccination.
- Planned receipt of immunoglobulins and/or any blood products within the three months preceding study enrollment or at any point during the study period until after the final safety telephone contact.
- Previous anaphylactic reaction, severe systemic response, or serious hypersensitivity to a prior immunization or a known allergy to synthetic ODNs, aluminum, formaldehyde, benzethonium chloride (phemerol), or latex.
- History of anthrax disease, suspected exposure to anthrax, or previous vaccination with any anthrax vaccine.
- Subject previously served in the military any time after 1990 and/or had planned to enlist in the military at any time from Screening through the final telephone contact.
- Acute disease at the time of enrollment (study allowed for subject rescreening for those with a transient acute condition according to procedures under Section 5.4 of the protocol [Appendix 16.1.1]).
- Any medical condition that, in the opinion of the investigator, adversely impacted the subject's participation or the conduct of the study.
- Major congenital defects or serious chronic illness, including any cancer other than the following: a) any non-metastatic cancer (excluding hematologic malignancies) or melanoma, of which the subject has been disease-free for at least five years; and b) localized skin cancer, resected (including squamous cell and basal cell carcinomas).
- A positive blood test for hepatitis B surface antigen (HBsAg), hepatitis C antibody, or human immunodeficiency virus (HIV) HIV-1 or HIV-2 antibodies.
- Chronic administration (defined as >14 days) of immunosuppressants or other immune-modifying drugs (included oral or parenteral corticosteroids, e.g., a glucocorticoid dose exceeding 10 mg/day prednisone or equivalent) within six months prior to the vaccine dose; inhaler use (e.g., for seasonal allergies) was permitted.
- Any confirmed or suspected immunodeficiency condition (congenital or secondary) or autoimmune disease based on medical history and PE.
- Family history of congenital or hereditary immunodeficiency.

Reviewer comment: *The Applicant's eligibility criteria were appropriate.*

Prohibited Concomitant Therapy

Prohibited and restricted concomitant medications during the study included anti-inflammatory or antipyretic medications, vaccines, immunomodulatory agents, antineoplastic agents, and immunoglobulins/other blood products. The complete list of prohibited and restricted concomitant therapies was provided in Table 5 of the CSR (STN 125761/0, EBS.AVA.212, CSR, Table 5, page 31 of 247).

6.1.4 Study Treatments or Agents Mandated by the Protocol

A summary of study treatments is provided in Table 2 in Section 6.1.2. AV7909 consists of AVA bulk drug substance derived from cell-free filtrates of microaerophilic cultures of an avirulent, nonencapsulated strain of *B. anthracis* and formulated with CpG 7909 adjuvant, a TLR9 agonist. Placebo (sterile, preservative-free saline) was administered SC on Day 29 for masking

purposes to subjects in Groups 1, 2, and 3. In this trial, only a single dose (0.5 mL) of AV7909 or matching placebo was used from each vial per subject.

Each group received a specific lot of AV7909 for a total of three AV7909 lots tested in Groups 1 to 3. The batch numbers for Groups 1 to 3 were Lots 100000A, 100001A, and 100002A, respectively, representing Lots 1, 2, and 3 in the study (these lots corresponded to bulk Lots 100000, 100001, and 100002). The batch number for Group 4 was Lot 300115A.

Sterile, preservative-free saline for injection (0.9% NaCl; (b) (4) ; lot number (b) (4) provided in 10 mL single-use vials was used for the placebo dose.

6.1.5 Directions for Use

IP was prepared and administered by an unblinded member of site staff following a physical examination (PE) and urine pregnancy test (if applicable). Vaccinations were administered in the deltoid region of alternating arms on Day 1, Day 15 (± 1 day), and Day 29 (± 2 days).

Subjects were observed for 30 minutes for adverse effects of vaccination, particularly signs and symptoms of anaphylaxis. The date and time of vaccine administration were recorded on source documents and the electronic case report form (eCRF).

6.1.6 Sites and Centers

The trial was conducted at 35 US sites.

Reviewer comment: *Data from study site US1027 were excluded from the data presented in the clinical memo due to the data integrity issues detailed in Section 3.2. Submission Quality and Completeness.*

6.1.7 Surveillance/Monitoring

Immunogenicity Evaluation

Functional (i.e., neutralizing) antibodies against LT were measured using the toxin neutralizing antibody (TNA) assay; blood samples were collected pre-vaccination on Day 1 (baseline) and on Days 29 and 64. Assay results were reported as the reciprocal of a serum sample dilution that resulted in 50% neutralization of the LT's cytotoxicity (50% effective dilution; ED₅₀). The results were divided by the ED₅₀ of a serum reference standard to standardize assay results, and the resulting ratio was reported as NF₅₀.

Reviewer comment: *The TNA assay used in this study for immunogenicity assessments was originally validated by the CDC to measure and quantify the functional ability of a serum specimen to neutralize *B. anthracis* LT activity using an *in vitro* cytotoxicity assay.³⁰ The assay was modified at (b) (4) to increase assay throughput from (b) (4) test samples per plate (high-throughput TNA assay). The high-throughput assay was validated for use with human serum at (b) (4).^{30,31} The validation provided documented evidence that the high-throughput assay consistently met the predetermined specifications and maintained the same level of quality over time. This same TNA assay was also used for licensure of BioThrax for the anthrax PEP indication under the Animal Rule (License 1755; STN 103821/5344).^{4,31,32}*

Lot consistency was assessed in this study by evaluating three consecutively

manufactured lots of AV7909 to determine if they induce a consistent antibody response in humans based on equivalence in the TNA NF₅₀ response at Day 64 across the three lots tested (Groups 1 to 3).

The summary of TNA NF₅₀ immune response by visit for each of the three lots of AV7909 (Groups 1-3), the three AV7909 lots pooled (Groups 1-3 combined), and the BioThrax group included:

- Descriptive summaries (n, mean, median, minimum, and maximum) of TNA NF₅₀ values,
- GMT and corresponding 95% CIs for TNA NF₅₀; calculated by taking the anti-logarithms of the mean and 95% CIs for log₁₀ TNA NF₅₀, and
- The percentage of subjects with TNA NF₅₀ ≥0.56 (also TNA NF₅₀ ≥0.29 and TNA NF₅₀ ≥0.15) with associated 95% CI (exact Clopper-Pearson confidence limit).

Safety Evaluation

Safety monitoring procedures for EBS.AVA.212 comprised the following:

- A complete PE at Screening and Day 64/Early Withdrawal Visit (EWV).
- Symptom-directed PE on Study Days 1, 15, and 29.
 - Abnormal PE findings recorded as medical history if these presented before the vaccination on Day 1 and as AEs after Day 1 post-vaccination if new or increased in severity/frequency following vaccination.
- Vital signs (VS; including systolic and diastolic blood pressure, heart rate, respiratory rate, and temperature) assessed at Screening and at each subsequent clinic visit (Days 1, 15, and 29) through Day 64/EWV, including unscheduled visits that occurred before Day 64.
 - Height and weight only recorded at Screening.
 - VS assessed prior to vaccination and at 30 ±5 minutes post-vaccination, to assess for adverse effects of vaccination, especially anaphylaxis.
- Concomitant medication use, with clinical indication (30 days prior to Screening till the last study visit [recorded on the eCRF]).
- Assessment of local and systemic reactogenicity and solicited AEs for at least seven days after each vaccination by study subjects using e-diaries (web-based diary card) with review of e-diaries one week after each vaccination. Study site staff reviewed the e-diary starting with the day following vaccination (Day 2), Day 15, and Day 29 (and the EWV if it fell within a window for e-diary entry). On Day 43 (±2 days), site staff followed up with a telephone call to review e-diary data entered by the subject after Day 29.
 - **Local reactogenicity** (injection site reactions) assessed using the following signs/symptoms: warmth, tenderness, itching, pain, arm motion limitation (AML), redness, induration, swelling, and bruising.
 - **Systemic reactogenicity** assessed using the following signs/symptoms: fatigue/tiredness, muscle ache, headache, and fever (oral temperature). Severity of warmth, tenderness, itching, pain, AML, induration/swelling, bruise (local reactogenicity), and fatigue/tiredness, muscle ache, and headache (systemic reactogenicity) graded using the following criteria: Grade 0=Symptom absent (within normal range or did not meet criteria for toxicity of at least Grade 1); Grade 1 [Mild]=symptom was present but did not interfere with activities of daily living; Grade 2 [Moderate]=symptom caused some interference with activities of daily living; Grade 3 [Severe]=symptom prevented activities of daily living or required treatment; Grade 4 [Potentially life-threatening]=symptom was potentially life-threatening and associated with a visit to an emergency room or hospitalization.

The toxicity grading scales (Grade 1 to 4) were based on the FDA Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (CBER, 2007)³³ and provided in Protocol EBS.AVA.212, Appendix A (Table 8; Appendix 16.1.1).

- Subjects assessed the diameter of erythema/redness and induration/swelling using the injection site measurement tool provided by the Applicant. Subjects used the following scale for assessing erythema and swelling:
 - 0 = Absent
 - 1 = Mild: 2.5 to 5 cm in diameter and does not interfere with activity
 - 2 = Moderate: 5.1 to 10 cm in diameter or interferes with activity
 - 3 = Severe: >10 cm in diameter or prevents daily activity
- Severity of temperature elevations was graded as follows:
 - Grade 0: no fever (<100.4 °F)
 - Grade 1: 100.4–101.1°F
 - Grade 2: 101.2–102.0°F
 - Grade 3: 102.1–104.0°F
- For a reaction not resolved at seven days postvaccination, subjects were to continue completing the e-diary daily until they were symptom free for two consecutive days.
- Study sites alerted of any ≥Grade 3 (e.g., ER visit or hospitalization) solicited systemic reactions which required discontinuation of vaccinations upon verification by the PI or designee. The PI/designee could require subjects reporting ≥Grade 3 reactions to return to the clinic for an unscheduled visit. Subjects were asked if they had taken pain/fever medications such as acetaminophen, aspirin, non-steroidal anti-inflammatory drugs, or other medication in the past 24 hours.
- Assessment of local and systemic reactogenicity in-clinic immediately before, and 30 minutes after vaccination.
 - In-clinic solicited injection site reactions (local and systemic) were the same as assessed by e-diary (i.e., warmth, tenderness, itching, pain, AML, redness, lump, swelling and bruise and fatigue/redness, muscle ache, headache, and fever).
 - Severity of solicited injection site and systemic reactions were evaluated in-clinic using the same toxicity grading scale as used in e-diary assessments (as provided in Appendix A of the study protocol).
 - Grade 3 solicited injection site and systemic reactions from in-clinic visits were also recorded on the AE eCRF, if confirmed by the PI.
- Unsolicited AEs, SAEs, and AESIs (Table 7, EBS.AVA.212, CSR, pages 40-42 of 247) evaluated during in-clinic (Day 1, 15, and 29) visits through Day 64 or EWV.
 - TEAEs were defined as AEs that presented after the initiation of treatment or any AEs already present that worsened in either intensity or frequency following treatment.
 - AESIs associated with autoimmune disease that could represent a safety signal for vaccine-associated autoimmunity were defined by CBER (Appendix B of the study protocol [Appendix 16.1.1]).
- Review of SAEs, AESIs, and medication use (only if related to a SAE and/or AESI) by telephone follow-up on Months 4, 7, 10, and 13 (i.e., 3, 6, 9, and 12 months after the last vaccination).
- Clinical laboratory testing (chemistry, hematology, CBC with differential) conducted at Screening and Day 29 (and EWV if EWV occurred before Day 29).
 - Female subjects were required to have a negative urine pregnancy test before receiving any vaccination (serum pregnancy test performed at Screening and Day

- 64/EWV; urine pregnancy test performed and status confirmed on Days 1, 15, and 29 prior to receipt of IP).
- Screening laboratory tests also included viral serology HIV antibodies, HBsAg, and hepatitis C antibody) and urine drug screen.
 - Auto-antibody testing (rheumatoid factor (RF), anti-nuclear antibody (ANA), anti-double-stranded deoxyribonucleic acid antibody (anti-dsDNA), and thyroid-stimulating hormone (TSH) levels) performed on Day 1 and 64 (or EWV).

From the time of signing the ICF until immediately before the first vaccination on Day 1, only AEs resulting from study-related procedures were recorded on the AE eCRF; all other events reported during this time interval were reported as 'Medical History'. AEs, SAEs, and AESIs (only confirmed AESIs by the Data Safety Monitoring Board [DSMB]) occurring from the time of the first vaccination on Day 1 up to Month 13, regardless of causal association with the IP, were recorded on the AE eCRF. The PI's (or designee's) assessment of an AE's causal relationship to the IP was documented on the AE eCRF using the following definitions: "unrelated," "possibly related," "probably related," and "definitely related" (Study Protocol for EBS.AVA.212, Section 9.2.2., page 79 of 109). If the relationship between the AE and the IP was determined to be "possibly related," "probably related," or "definitely related," the event was considered related to IP.

All AEs, SAEs, and AESIs were followed until resolution, stabilization, or up to 30 days after the last study visit (Month 13), unless the subject withdrew consent, was lost to follow-up, or was referred for appropriate long-term medical care.

Reviewer comment: *EBS.AVA.212's safety monitoring plan appeared to be carried out appropriately. Solicited local and systemic injection site reactions in EBS.AVA.212 were evaluated in essentially the same manner as performed during assessment of BioThrax for the PEP indication in the pivotal Phase 3 Study EBS.AVA.006 that supported licensure of BioThrax for PEP (STN 103821/5344). The severity of solicited and unsolicited AEs, laboratory tests, and VS was assessed based on the FDA Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials.³³ Toxicity severity criteria in EBS.AVA.212 were identical to those used in the assessment of solicited and unsolicited AEs, laboratory tests, and VS for BioThrax, in Study EBS.AVA.006.*

Baseline autoimmune panel laboratory tests provided laboratory evidence required to determine whether a condition of autoimmune etiology was pre-existing or new onset; thus, these tests were incorporated in this Phase 3 trial to provide a baseline reference to inform the causality assessment of AESIs to IP administration in the event of a confirmed autoimmune disease diagnosis. Baseline autoimmune panel results were not intended for determination of a subject's initial or continued study eligibility.

Adverse Events of Special Interest (AESIs)

During telephone follow-up calls to assess safety, staff members were to elicit and record any information on AESIs of potential autoimmune etiology in the source documentation. For conditions that were diagnosed or suspected AESIs, staff members were to refer the subject to the PI or designee for further phone evaluation. Subjects were asked to return for an unscheduled clinic visit for evaluation (Section 8.1.8) and to provide a blood sample for auto-antibody testing and/or TSH assessment, if needed. The PI or designee was to obtain records confirming the diagnosis or refer the subject to a medical specialist for additional clinical testing and follow up until the diagnosis was confirmed or negated. Potential AESIs were to be reported

to the Applicant immediately, as specified in Section 9.3, and followed up per procedures described in Section 9.6.2 of the study protocol. If the DSMB assessed the case as a confirmed AESI, the PI or designee was directed to record the occurrence of the AESI on the AE eCRF along with any medications taken. The Medical Monitor (MM) or designee was to direct the completion of the AE eCRF when communicating the DSMB assessment outcome(s) to the PI or designee.

Reviewer comment: *The Applicant's AESI monitoring procedures were appropriate.*

Data Safety Monitoring Board (DSMB)

Independent safety oversight was provided by a DSMB, which was notified of significant AEs (e.g., SAEs, severe AEs recorded on the eCRF, potential AESIs of autoimmune etiology, or any other relevant events; refer to Section 8.4.1 of the protocol [Appendix 16.1.1]) by the MM on an ongoing basis.

The DSMB comprised at least three voting members, including one expert in immunology (i.e., rheumatologist, immunologist) to specifically support the evaluation of AESIs. This member reviewed, on a blinded basis, all potential AESIs to assess cases for autoimmune etiology, if pre-existing or new onset, and their relationship to IP administration. A planned interim DSMB safety data review was conducted after the first 500 subjects completed the Day 29 visit, comprising all safety evaluations two weeks after the second vaccination. All DSMB reviews were performed with blinded data unless otherwise requested by the DSMB Chair. The DSMB made recommendations regarding the safety of continuing enrollment and dosing, or study termination.

Study enrollment and vaccinations could be temporarily halted by the DSMB or the Applicant if any of the following occurred, pending further evaluation:

- >3% of subjects had the same Grade 3 or higher AE.
- 3 suspected unexpected serious adverse reactions (SUSARs) reported within the same body system, as assessed by the Applicant.
- 5 potential AESIs considered related to the IP reported, as assessed by the Applicant.
- A single AESI considered related to the IP reported, as assessed by the DSMB.

The operations of the DSMB were detailed in a DSMB charter which was finalized prior to screening the first subject (DSMB charter provided in Section 8.4.1 of EBS.AVA.212 Study Protocol).

Reviewer comment: *The DSMB appeared to have appropriately conducted AE assessment and determination to continue Study 212, based on the DSMB charter.*

6.1.8 Endpoints and Criteria for Study Success

Primary Endpoints:

Two sets of primary immunogenicity endpoints were evaluated; one to establish lot consistency, the other to demonstrate immunogenicity of AV7909 at a clinically relevant time point (Day 64). Each respective endpoint consisted of two co-primary endpoints, tested hierarchically, as follows:

1. Demonstration of Lot-to-lot Consistency of AV7909:

Co-Primary immunogenicity endpoints:

a. GMT Ratio of TNA NF_{50} at Day 64.

Success Criteria: This endpoint was met if the 95% CIs for the Day 64 TNA NF_{50} GMT ratios between all three pairs of AV7909 groups (Lot 1 versus (vs.) Lot 2, Lot 2 vs. Lot 3, and Lot 1 vs. Lot 3) were within 0.5 and 2.0.

b. Assessment of the percentage of subjects reaching the threshold of protection (defined as a TNA NF_{50} of ≥ 0.56) at Day 64 in each of the three AV7909 lots.

Success Criteria: The protective level of immunogenicity in all three AV7909 lots was demonstrated if the lower bound (LB) of the 2-sided 95% CI is $\geq 40\%$ for the percentage of AV7909 subjects in each of the three lots achieving a TNA $NF_{50} \geq 0.56$ at Day 64.

2. AV7909 Immunogenicity at Day 64:

Co-Primary immunogenicity endpoints:

a. Percentage of subjects with a protective threshold, as defined by a TNA NF_{50} value of ≥ 0.56 at Day 64 for the three pooled AV7909 lots.

Success Criteria: AV7909 was considered to have achieved a protective level of immunity under the Animal Rule at Day 64 if the LB for the 2-sided 95% CI for the percentage of subjects with TNA NF_{50} values above the specified threshold of protection (≥ 0.56) was $\geq 40\%$.

b. Non-inferiority of AV7909 to BioThrax

Success Criteria: Non-inferiority demonstrated if the LB of the 2-sided 95% lower CI for the difference in the percentage of subjects (AV7909 – BioThrax) achieving the Day 64 TNA $NF_{50} \geq 0.29$ (TNA threshold) was above -15%.

Reviewer comment: *The first co-primary immunogenicity endpoint (the percentage of subjects with a TNA NF_{50} value of ≥ 0.56 at Day 64 for the three pooled AV7909 lots) is a threshold of protection derived from animal studies (TNA NF_{50} values), which bridges observed immunogenicity responses and survival rates in vaccinated animals after anthrax challenge to observed immunogenicity responses in humans. It represents the antibody response at Day 64 in humans administered AV7909 that was determined as protective in animals (primary endpoint).⁴ Day 64 was the anticipated time point at which concomitant antimicrobial therapy was to be completed, which is a time of heightened risk for humans for susceptibility to residual anthrax spores.*

The TNA NF_{50} 0.56 threshold was included as a component of the co-primary immunogenicity endpoint in pivotal study EBS.AVA.212 per CBER request in an Agency letter dated February 10, 2016; as it was part of the basis for licensure of BioThrax for anthrax PEP and represents the highest protective threshold value from three species (rabbits, guinea pigs, and NHPs) evaluated with BioThrax or AV7909.

The second co-primary immunogenicity endpoint, defined as the non-inferiority of the antibody response of AV7909 to AVA at Day 64, represents antibody levels determined to be protective in animals administered BioThrax, the only vaccine currently licensed in the US for anthrax PEP, which is a component of AV7909 vaccine. The success criterion for this endpoint to establish Day 64 protective immunity in humans (i.e., LB of the two-sided 95% CI for the percent of subjects achieving the threshold being greater than or equal to 40%) was based on the BioThrax PEP clinical development program, where 40% was utilized as a conservative measure corresponding to adequate vaccine efficacy (VE).³⁴

The TNA threshold of 0.29 is based on BioThrax NHP Study 844, as described in the BioThrax USPI.¹ Because rabbits do not respond to CpG adjuvant, the NHP is the only animal species in which the thresholds of protection have been evaluated for both BioThrax and AV7909. AV7909 NHP Study No. 3655-100072763, in which AV7909 was administered by the Day 0 and 14 vaccination schedule, with challenge at Day 70; resulted in a similar threshold value (0.26). In NHPs, a TNA NF₅₀ value ≥ 0.29 was associated with similar levels of protection ($\geq 70\%$) for both BioThrax and AV7909. Therefore, this threshold value was deemed acceptable by FDA to provide the basis for comparing putative effectiveness of BioThrax and AV7909 in humans (IND 14451.A78). The percentage of subjects above the protective TNA threshold of 0.29 was determined to be an appropriate the endpoint by FDA for bridging animal efficacy data to human immunogenicity data per the Animal Rule.

In summary, FDA's decision to require a co-primary endpoint of non-inferiority was made to comply with the Animal Rule and immunobridge human immunogenicity data to a second animal species (FDA Phase 3 Comments, March 29, 2016). Use of 0.29 as the TNA NF₅₀ threshold was acceptable because it was supported by the NHP study and because the more conservative value of 0.56 (rabbit threshold) was incorporated in the other co-primary immunogenicity endpoint. Use of the 0.29 threshold was additionally supported by repeat rabbit pre-exposure studies which showed TNA NF₅₀ protective thresholds ranging from 0.19 to 0.29 that correlated with 70% rabbit survival rather than the 0.56 threshold observed in the initial rabbit challenge study (see the ES in clinical review and Dr. Tod Merkel's nonclinical review for more details).

Safety:

- Incidence of SAEs from the time of the first vaccination on Day 1 through the 12-month safety follow-up telephone call following the last vaccination.

Secondary Endpoints:

Immunogenicity:

- LB of the 2-sided 95% CI was to be $\geq 67\%$ for the percentage of AV7909 subjects in Groups 1 through 3 (3 lots pooled), achieving a TNA NF₅₀ ≥ 0.15 on Day 29.

Success Criteria: Day 29 protective immunity of AV7909 in humans established if the LB of the 2-sided 95% CI for the percentage of subjects achieving threshold was $\geq 67\%$.

Reviewer comment: *The threshold of protection (0.15) for the secondary immunogenicity endpoint was based on the Day 28 TNA NF₅₀ value associated with 70% survival in NHPs administered AV7909 on Days 0 and 14 and challenged with B. anthracis spores on Day 28.³⁵ The more stringent criterion of TNA NF₅₀ at Day 29 was selected as a secondary endpoint since demonstration of protection on Day 29 after vaccination with AV7909 could potentially mitigate the risk associated with the lack of compliance with concomitant prophylactic antimicrobial therapy.*

Safety:

- Incidence of solicited systemic reactions and solicited injection site reactions, by severity, following each vaccination as reported in subject e-diaries.
- Incidence of AEs from the time of the first vaccination on Day 1 through Day 64.
- Incidence of clinical laboratory test abnormalities.
- Incidence of AESIs from the time of the first vaccination on Day 1 through the 12-month safety follow-up telephone call following the last vaccination.

6.1.9 Statistical Considerations & Statistical Analysis Plan

For a detailed discussion of the statistical analysis plan (SAP), the clinical reviewer refers to the statistical review of EBS.AVA.212 for BLA STN 125761/0.

In summary, the sample size for Study EBS.AVA.212 was based primarily on safety considerations. The total sample size across all three AV7909 study groups was based on 3300 subjects. Allowing a 10% drop-out rate, the sample size for safety (3000) was deemed sufficient to detect, with 95% probability, an AE rate of 1:1000, or 0.1%.

It was assumed that the lot-to-lot GMT ratio could be as low as 0.6 based on the Applicant's experience with BioThrax. The largest GMT ratio between two out of three lots was conservatively assumed to be 1.5. Given a coefficient of variation (CV) of 100% (slightly larger than the observed 91% in the Phase 2 Study EBS.AVA.208, EBS.AVA.212 had >99% power to demonstrate lot consistency with the prespecified equivalence bounds ([0.5, 2.0]) for GMT ratio of TNA NF₅₀ at Day 64.

Sample sizes of 400 and 2400 provided approximately 98% power to demonstrate non-inferiority of the two-dose AV7909 IM regimen to the three-dose BioThrax SC regimen at Day 64 with a non-inferiority margin of -15%.

All immunogenicity and lot consistency analyses were based on the PP Population. No imputation was made for missing data.

All safety analyses were performed based on the Safety Population (see definitions under Section 6.1.10.1 Populations Enrolled/Analyzed, below). Data summaries were tabulated by treatment group (AV7909 (three lots pooled) and BioThrax group) as specified in Section 3.6 of the SAP (Appendix 16.1.9). Medical history and AEs (TEAEs and SAEs) were coded to System Organ Class (SOC) and Preferred Terms (PTs) using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary version 22.0.

To evaluate the consistency of immunogenicity results and safety profiles across subgroups, immunogenicity and safety results were summarized for AV7909 (three lots pooled) and BioThrax groups by age, sex, and race. No formal statistical hypothesis testing was performed (EBS.AVA.212, SAP, Version 2.0, September 15, 2020, Section 4.6 Subgroup Analysis, page 23 of 51).

Changes in the Conduct of the Study or Planned Analyses:

The original protocol dated September 14, 2018 (Version 1.0), was amended five times. All subjects were enrolled under the final protocol (Version 4.2) which comprises the study design described above.

Changes in the Planned Analyses

FDA requested re-analysis of safety data excluding data from site US1027 for reasons previously described in the memorandum; the Applicant provided immunogenicity and safety analyses excluding data from that site. A sensitivity analysis was performed by the Applicant excluding site US1027. All analyses immunogenicity and safety analyses presented in this review exclude site US1027; no conclusions changed as a result of this reanalysis.

In addition to the revised ADFACE dataset, corrected source tables were provided for solicited reactions for Study EBS.AVA.212.

Reviewer comment: *Except where otherwise noted, all data analyses (demographics, subject disposition, protocol violations, immunogenicity, and safety) are presented with study site US1027 excluded and based on revised CE and AE and corrected ADFACE and ADAE datasets (EBS.AVA.212 CSR Addendum 3) submitted under STN 125761/0/27 and 125761/0/38, respectively.*

6.1.10 Study Population and Disposition

A total of 3862 subjects were randomized in EBS.AVA.212, with 173 subjects excluded from study site US1027 (n=3689 total randomized excluding site US1027). The resultant randomized 3156 subjects in EBS.AVA.212 were allocated to the three AV7909 lots in comparable numbers: 1053 subjects (100.0%) in Lot 1, 1054 subjects (100.0%) in Lot 2 and 1049 subjects in Lot 3 (100.0%; n=3156 in the three pooled AV7909 lots). There were 533 subjects who were randomized to the BioThrax (AVA) group.

Reviewer comment: *A similar number of subjects were randomized to the three AV7909 lots.*

6.1.10.1 Populations Enrolled/Analyzed

There were three analysis populations for this study:

- The intent-to-treat (ITT) Population included all randomized subjects. Subjects were included in the study group to which they were randomized.
- The Safety Population included all randomized subjects who received at least one vaccination. According to the vaccine received, safety analyses were based on the Safety Population (BioThrax and the combined AV7909 groups).
- The PP Population included subjects who were randomized and did not have any of the protocol deviations listed below:
 - History of previous anthrax disease, anthrax exposure, or anthrax vaccination as per eligibility criteria, as evidenced by a baseline (Day 1 pre-vaccination) TNA NF₅₀ above the limit of detection,
 - Missing or out-of-window vaccination visit at Study Day 15,
 - Missing or out-of-window vaccination visit at Study Day 29 for the BioThrax group,
 - Administration issue(s) with IP, e.g., an incorrect dose of IP at one or more vaccination visits, administration of IP associated with a temperature excursion,
 - Use of prohibited or restricted medications which could have impacted immune response to vaccination as assessed by the Applicant (this assessment was completed prior to database lock),
 - Missing immunogenicity data (e.g., sample out-of-window, sample not shipped/received, sample not usable by the immunogenicity lab, sample associated with loss of cold chain) at Day 64.

The PP Population was used for analyses of lot consistency and immunogenicity. Subjects were included in the study group based on the vaccine received.

As shown in Table 3 below, the Safety and PP Populations comprised 99.9% (n=3684) and 80.6% (n=2973), respectively, of the ITT population.

Table 3. EBS.AVA.212: Analysis Populations

Analysis Population ¹	AV7909 Lot 1 n (%)	AV7909 Lot 2 n (%)	AV7909 Lot 3 n (%)	AV7909 (Three Lots Pooled) n (%)	BioThrax n (%)
Intent-to-Treat (ITT)	1053 (100.0)	1054 (100.0)	1049 (100.0)	3156 (100.0)	533 (100.0)
Safety	1050 (99.7)	1053 (>99.9)	1048 (>99.9)	3151 (99.8)	533 (100.0)
Per Protocol (Immunogenicity)	835 (79.3)	854 (81.0)	854 (81.4)	2543 (80.6)	430 (80.7)

n = number of subjects; % = percentage of subjects based on number of randomized subjects

¹All analysis populations exclude study site US1027.

Ref: STN 125761/0, EBS.AVA.212, CSR Addendum 2, Table 12, page 18 of 73; Source: Table 14.1.1.2b.

Reviewer comment: *The proportion of randomized subjects in the PP Population across treatment groups was numerically similar, with approximately 20% of randomized subjects excluded from the PP population due to protocol deviations.*

6.1.10.1.1 Demographics

Subject demographics were assessed by age (18-30, 31-50, and 51-65 years), sex (female, WOCBP, male), race (White, Black or African American, and Other/More than One Race), and ethnicity (Not Latino/Hispanic, Hispanic/Latino, Unknown, and Not reported).

There were no meaningful across-treatment differences observed in the subject demographics. A somewhat higher proportion of younger (31-50 years; 44.4%) and female subjects (57.5%) were seen across treatment groups. The majority of subjects across all treatment groups were White (2868 subjects, 77.9%) and non-Hispanic/non-Latino (3071 subjects, 83.4%).

A summary of subject baseline characteristics (height, weight, and BMI) indicate that baseline characteristics were likewise similar across treatment groups. The overall mean (standard deviation; SD) height and weight of subjects were 170.4 (9.8) cm and 79.1 (15.7) kg, respectively. The mean BMI across groups was very similar (range 27.0-27.3).

Reviewer comment: *There was no appreciable difference in subject demographic or baseline characteristics seen across the study groups for EBS.AVA.212.*

6.1.10.1.2 Medical/Behavioral Characterization of the Enrolled Population

Concomitant Medical Conditions

Most subjects (77.9%; 3006/3857 subjects from the original CSR for EBS.AVA.212) had at least one concomitant medical condition; the proportion of subjects with at least one medical history finding was similar between the combined AV7909 group (78.1%; 2576/3299) and the BioThrax group (77.1%; 430/558). The most common concomitant medical condition reported was seasonal allergy (21.3%; 823/3857), with all other medical conditions observed in <10% of the Safety Population.

Concomitant Medication Use

The most frequently used medications (≥5% subjects/group) for subjects in the combined AV7909 group and BioThrax group (all study sites; EBS.AVA.212, CSR) were propionic acid derivatives, e.g., ibuprofen or naproxen (28.6% and 22.6%, respectively) and anilides, e.g., paracetamol (15.1% and 16.1%, respectively). There was no meaningful difference in the proportion of subjects who had concomitant medications in the combined AV7909 group (70.3%; 2318/3299) compared with the BioThrax group (67.2%; 375/558).

Reviewer comment: *The frequency of concomitant medication use was generally similar across treatment groups. No difference in medical/behavioral characteristics of the enrolled population or imbalances across treatment groups were identified in EBS.AVA.212 that might affect (or theoretically affect) the study’s immunogenicity and safety results.*

6.1.10.1.3 Subject Disposition

A total of 3689 healthy adults meeting eligibility criteria were randomized to one of the four treatment groups, as shown in Table 4 below.

Table 4. Study EBS.AVA.212: Subject Disposition (ITT Population)

Disposition¹	AV7909 Lot 1 n (%)	AV7909 Lot 2 n (%)	AV7909 Lot 3 n (%)	AV7909 (3 Lots Pooled) n (%)	AVA (BioThrax) n (%)
Randomized (n)	1053	1054	1049	3156	533
Not Treated (n)	3 (0.3)	1 (<0.1)	1 (<0.1)	5 (0.2)	0
Treated (n)	1050 (99.7)	1053 (>99.9)	1048 (>99.9)	3151 (99.8)	533 (100.0)
Completed study treatment (received all 3 study vaccinations)	899 (85.4)	921 (87.4)	911 (86.8)	2731 (86.5)	472 (88.6)
Discontinued study (did not receive all study vaccinations)	151 (14.3)	132 (12.5)	137 (13.1)	420 (13.3)	61 (11.4)
Completed 12-month safety follow-up (Day 394 follow-up)	984 (93.4)	977 (92.7)	987 (94.1)	2948 (93.4)	512 (96.1)
Did not complete 12-month safety follow-up (study withdrawal)	66 (6.3)	76 (7.2)	61 (5.8)	203 (6.4)	21 (3.9)
Primary reason for treatment discontinuation:	--	--	--	--	--
Adverse Event	30 (2.8)	35 (3.3)	34 (3.2)	99 (3.1)	15 (2.8)
Death	0	0	1 (<0.1)	1 (<0.1)	0
Withdrawal by Subject	25 (2.4)	20 (1.9)	20 (1.9)	65 (2.1)	4 (0.8)
Lost to follow-up	6 (0.6)	7 (0.7)	5 (0.5)	18 (0.6)	4 (0.8)
Other ²	90 (8.5)	70 (6.6)	77 (7.3)	237 (7.5)	38 (7.1)
Primary reason for study withdrawal:	--	--	--	--	--
Adverse Event	1 (<0.1)	0	0	1 (<0.1)	0
Death	2 (0.2)	2 (0.2)	2 (0.2)	6 (0.2)	0
Lost to Follow-up	41 (3.9)	52 (4.9)	45 (4.3)	138 (4.4)	17 (3.2)
Non-compliance with study drug	0	0	0	0	0
Physician decision	2 (0.2)	0	2 (0.2)	4 (0.1)	0

Disposition ¹	AV7909 Lot 1 n (%)	AV7909 Lot 2 n (%)	AV7909 Lot 3 n (%)	AV7909 (3 Lots Pooled) n (%)	AVA (BioThrax) n (%)
Withdrawal by subject	16 (1.5)	21 (2.0)	11 (1.0)	48 (1.5)	4 (0.8)

n = number of subjects; % = percentage based on number of randomized subjects.

¹Site US1027 excluded from the assessment of subject disposition. Four subjects in the study were found to have enrolled (vaccinated) twice at two clinical study sites that were in close proximity to one another. The subjects were withdrawn from the study at the second enrollment site at the time of discovery and completed the study at the first enrollment site. Subjects' disposition status at the first enrollment site are displayed in this table.

²'Other' reason for treatment discontinuation defined as 'Visit would have been out of treatment window.'

Ref: STN 125761/0, EBS.AVA.212, CSR Addendum 2, Table 1, pages 13-14 of 73, Figure 1, page 15 of 73.

Source: Table 14.1.1.1b; Listing 16.2.13

The majority of subjects (>85%) in all study groups completed all three vaccinations in accordance with the protocol, which included vaccinations given without any administration issues; most subjects also completed the 12-month safety follow-up (>92%). The proportion of subjects who received all three vaccinations was similar between the combined AV7909 group and the BioThrax group. The most common reason for discontinuation of treatment was the study visit being out of treatment window (i.e., also defined as 'Other'; 6.6-8.5% across all treatment groups), followed by an AE (2.8-3.3% across all treatment groups), followed by subject withdrawal (0.8-2.4% across all treatment groups).

Reviewer comment: *Compliance with vaccination was high across all treatment groups. Most subjects completed the study, including the 12-month safety follow-up after the final vaccination.*

Evaluation of protocol deviations for all randomized subjects (ITT population) indicate that the proportion of subjects across all treatment groups with any protocol deviation (critical, major, or minor) was approximately 80-85%, though most protocol deviations were considered minor (71-77%). The proportion of subjects across treatment groups with critical deviations were approximately 2-2.8%, with inappropriate eligibility and entry criteria being the most common critical protocol deviation. The proportion of subjects across treatment groups with 'major' protocol deviations was approximately 37-39%, with 'other criteria', followed by 'study procedures' accounting for most 'major' protocol deviations. The Applicant clarified the definition of 'other criteria' for major and minor protocol deviations as protocol deviations primarily related to subject e-diary compliance (STN 125761/0/42, submitted April 4, 2023). Major protocol deviations of 'other criteria' pertaining to e-diary compliance were defined as missing e-diary entries for ≥ 4 days, while minor e-diary protocol deviations were defined as missing e-diary entries for ≤ 3 days. All protocol deviations were determined prior to subject unblinding.

Reviewer comment: *The proportion of subjects with protocol deviations was high across all treatment groups, but balanced—with no study group accounting for the majority of protocol deviations. An explanation for the high proportion of subjects with protocol deviations across all treatment groups was not provided by the Applicant. It is plausible that in part, protocol deviations may have been more commonly seen in this study because of its long duration and greater likelihood for subjects missing required study windows for study visits and procedures, such as e-diary compliance and laboratory testing—findings that were documented in the study report and corresponding subject line listings for EBS.AVA.212.*

6.1.11 Efficacy Analyses

The TNA assay was performed on blood samples collected pre-vaccination on Days 1 (baseline), 29 and 64, with results reported as NF_{50} . All efficacy (immunogenicity) analyses were carried out using the PP Population.

6.1.11.1 Analyses of Primary Endpoint(s)

The primary immunogenicity endpoints for Study EBS.AVA.212 comprised two sets of endpoints: the first to establish AV7909 lot consistency of three consecutive AV7909 lots and the second, to demonstrate AV7909 immunogenicity at Day 64 (see 1 and 2 below). Each respective endpoint consisted of two co-primary endpoints, as follows:

1. Demonstration of lot-to-lot consistency of AV7909:
 - a. Co-Primary immunogenicity endpoints:
 - i. GMT Ratio of TNA NF_{50} at Day 64.
 - ii. Assessment of the percentage of subjects reaching the threshold of protection (TNA NF_{50} of ≥ 0.56) at Day 64 in each of the three AV7909 lots.
2. AV7909 Immunogenicity at Day 64:
 - a. Co-Primary immunogenicity endpoints:
 - i. Percentage of subjects with a protective threshold, as defined by a TNA NF_{50} value of ≥ 0.56 at Day 64 for the three pooled AV7909 lots.
 1. AV7909 was considered to have achieved a protective level of immunity under the Animal Rule at Day 64 if the LB for the two-sided 95% CI for the percentage of subjects with TNA NF_{50} values above the specified threshold of protection (≥ 0.56) was $\geq 40\%$.
 - ii. Non-inferiority of AV7909 to BioThrax
 1. Non-inferiority demonstrated if the LB of the 2-sided 95% lower CI for the difference in the percentage of subjects (AV7909 – BioThrax) achieving the Day 64 TNA $NF_{50} \geq 0.29$ (TNA threshold) was above -15%.

The primary immunogenicity endpoints were tested hierarchically, with testing of the next endpoint performed only if the previous endpoint met prespecified success criteria (i.e., demonstration of AV7909 lot consistency prior to evaluation of AV7909 immunogenicity). The overall type I error rate was controlled at less than 5% and no additional adjustment was needed.

Demonstration of Lot Consistency

Consecutive evaluation of the two co-primary immunogenicity endpoints for lot-to-lot consistency is summarized as follows:

1. AV7909 Lot Consistency Based on GMT Ratio of TNA NF_{50} at Day 64 (First Co-Primary Endpoint for Lot Consistency)

Results of the first co-primary immunogenicity endpoint for lot consistency are shown in Table 5 below.

Table 5. Study EBS.AVA.212: Lot Consistency Evaluated with Geometric Mean Titer Ratios of TNA NF₅₀ between Three AV7909 Lots at Day 64 (PP Population¹)

AV7909	Lot 1/Lot 2	Lot 1/Lot 3 Lot 2	Lot 2/Lot 3
GMT Ratio of TNA NF ₅₀ ²	1.03	1.07	1.04
95% CI of GMT Ratio ³	0.94, 1.13	0.98, 1.17	0.95, 1.13

GMT=Geometric Mean Titer; TNA=Toxin-neutralizing antibody; NF₅₀=50% neutralization factor TNA NF₅₀ values below the lower limit of quantitation (LLOQ) imputed as 0.032, which is half of the LLOQ of the assay. CI=Confidence interval

¹PP Population excludes site US1027.

²The linear regression model was fitted with log₁₀ NF₅₀ (Day 64) as the dependent variable and lot as the independent variable (categorical), assuming common residual variance. The mean differences in log₁₀ NF₅₀ between all pairs of lots with 95% CIs were transformed (anti-log) back to the scale of GMT ratios.

³AV7909 was considered as having achieved the pre-specified equivalent immunogenicity criterion if the 95% CI of GMT ratio fell within the equivalence range of [0.5, 2.0] for all three pairs of lot comparison at Day 64.

Ref: STN 125761/0, EBS.AVA.212, CSR Addendum 2, Table 6, page 22 of 73. Source: Table 14.2.2b.

For each of the three lot-to-lot comparisons, the 95% CI for ratio of GMT TNA NF₅₀ at Day 64 was found to be within the pre-defined criteria of 0.5 and 2.0, indicating equivalent immunogenicity across the three consecutive AV7909 lots.

Reviewer comment: Results of the lot consistency analysis (GMT Ratios of TNA NF₅₀ between three AV7909 lots at Day 64; including the 95%CI of the GMT Ratio) for all study sites (STN 125761/0, EBS.AVA.212, CSR, Table 16, page 70 of 247. Source: Table 14.2.2.) vs. study site US1027 excluded (STN 125761/0, EBS.AVA.212 CSR Addendum 2, Table 6, page 22 of 73. Source: Table 14.2.2b) indicated a negligible numerical difference in the GMT Ratios of TNA NF₅₀ for the different lot comparisons (data not shown)—indicating that exclusion of site US 1027 did not alter the results or conclusions regarding this first co-primary immunogenicity endpoint for AV7909 lot-to-lot consistency.

2. AV7909 Lot Consistency Based on Protective Level of Immunogenicity at Day 64 (Second Co-Primary Endpoint for Lot Consistency)

The second lot consistency co-primary immunogenicity endpoint tested was the threshold of protection (TNA NF₅₀ of ≥0.56) at Day 64 in all three AV7909 lots.

A total of 1685 (66.3%) subjects in the combined AV7909 group (three lots pooled) achieved a TNA NF₅₀ of ≥0.56 at Day 64 (See Table 6 below); these results were identical to those seen with site US1027 included (n=1771, 66.3%; data not shown; STN 125761/0, EBS.AVA.212, CSR, Table 17, page 70 of 247. Source: Table 14.2.3) indicating that exclusion of site US1027 did not alter the immunogenicity findings for this endpoint.

Table 6. Study EBS.AVA.212: Lot Consistency and Immunogenicity of AV7909 Evaluated with Percent of Subjects with TNA NF₅₀ ≥0.56 at Day 64 (PP Population¹)

Percent of Subjects with TNA NF ₅₀ ≥0.56 at Day 64	AV7909 Lot 1 (N=835)	AV7909 Lot 2 (N=854)	AV7909 Lot 3 (N=854)	AV7909 (Three Lots Pooled) (N= 2543)
n, Percent (%)	575 (68.9)	560 (65.6)	550 (64.4)	1685 (66.3)
95% CI ²	65.6, 72.0	62.3, 68.8	61.1, 67.6	64.4, 68.1

TNA = Toxin-neutralizing antibody; NF₅₀ = 50% neutralization factor; N = Number of subjects in each treatment arm in the PP Population; n, Percent (%) =Number and percentage of subjects achieving a TNA NF₅₀ cut-off value based on Per-Protocol population; CI = confidence interval

TNA NF₅₀ values below the lower limit of quantitation (LLOQ) were imputed as 0.032, which was half of the LLOQ of the assay. The 95% CI was based on the exact Clopper-Pearson confidence limit.

¹PP Population excludes site US1027.

²Lot consistency (protective level of immunogenicity) in all three AV7909 lots demonstrated if the lower bound of the two-sided 95% CI is ≥40% for the percentage of AV7909 subjects in each of the 3 lots achieving a TNA NF₅₀ ≥0.56 at Day 64.

Ref: STN 125761/0, EBS.AVA.212, CSR Addendum 2, Table 7, page 23 of 73. Source: Table 14.2.3b.

The LB 95% CI of the percentage of AV7909-treated subjects in each of the three AV7909 lots who achieved a TNA NF₅₀ ≥0.56 at Day 64 was greater than the pre-defined criterion of ≥40% in each AV7909 lot; therefore, it was concluded that a protective level of immunogenicity was achieved in all three AV7909 lots.

Reviewer comment: Success criteria for both lot consistency immunogenicity endpoints were met, which indicated that the AV7909 manufacturing process appeared consistent across AV7909 lots.

AV7909 Immunogenicity at Day 64:

To demonstrate AV7909 immunogenicity, two co-primary immunogenicity endpoints were evaluated consecutively.

1. The first co-primary immunogenicity endpoint tested was an assessment of the percentage of subjects with TNA NF₅₀ ≥0.56 at Day 64 from the three pooled AV7909 lots. This result is summarized in Table 7 below.

Table 7. Study EBS.AVA.212: AV7909 Immunogenicity of the Three Pooled AV7909 Lots compared to BioThrax, as Defined by the Percentage of Subjects Achieving a TNA NF₅₀ value of ≥0.56 at Day 64 (PP Population¹)

Percent of Subjects with TNA NF ₅₀ ≥0.56 at Day 64	AV7909 Lot 1 (N=878)	AV7909 Lot 2 (N=896)	AV7909 Lot 3 (N=896)	AV7909 (Three Lots Pooled) (N=2670)	BioThrax (N=454)
n	835	854	854	2543	430
GMT	0.765	0.741	0.716	0.740	0.330
Lower 95% CI	0.718	0.698	0.673	0.714	0.299
Upper 95% CI	0.814	0.788	0.762	0.767	0.363
n, Percent (%) of subjects with TNA NF ₅₀ ≥0.56	575 (68.9%)	560 (65.6%)	550 (64.4%)	1685 (66.3%)	134 (31.2)
95% CI ²	65.6, 72.0	62.3, 68.8	61.1, 67.6	64.4, 68.1	26.8, 35.8

TNA = Toxin-neutralizing antibody; NF₅₀ = 50% neutralization factor; N = Number of subjects per study group in the Per-Protocol population; n, Percent (%) = Number and percentage of subjects achieving a TNA NF₅₀ cut-off value based on Per-Protocol population

GMT = Geometric mean titer

TNA NF₅₀ LLOQ = 0.064. TNA NF₅₀ values below the lower limit of quantitation (LLOQ) were imputed as 0.032 in the calculations of Mean/SD/GMT, which was half of the LLOQ of the assay. CI = Confidence interval

¹PP Population excluded site US1027.

²The 95% CI was based on the exact Clopper-Pearson confidence limit. AV7909 was considered to have achieved a protective level of immunity per the Animal Rule at Day 64 if the lower bound for the two-sided 95% CI for the percentage of subjects with TNA NF₅₀ values above the specified threshold of protection (≥0.56) was ≥40%.

Ref: STN 125761/0, EBS.AVA.212 CSR Addendum 2, Table 8, page 24 of 73. Source: Table 14.2.1b

Per Table 7 above, the LB of the two-sided 95% CI was ≥40% for the combined (three lots pooled) AV7909 group, thereby meeting success criteria for this endpoint. Negligible differences in the percentage of AV7909 subjects achieving the protective threshold were seen between subjects from all study sites (data not shown).

2. Non-inferiority of AV7909 to BioThrax

The second co-primary AV7909 immunogenicity endpoint tested was the non-inferiority of AV7909 to BioThrax at Day 64, excluding site US1027, as shown in Table 8 below.

Table 8. Study EBS.AVA.212: Comparison of the Percentage of Subjects with a TNA NF₅₀ ≥0.29 for AV7909 versus BioThrax at Day 64 (PP Population¹)

Percent of Subjects with TNA NF ₅₀ ≥0.29 at Day 64	AV7909 (Three Lots Pooled) (N=2543)	BioThrax (N=430)
n, Percent (%) with TNA NF ₅₀ ≥0.29	2203 (86.6)	264 (61.4)
95% CI ²	85.2, 87.9	56.6, 66.0

N = Number of subjects per study group in the Per-Protocol population; n (%) = Number and percentage of subjects achieving a TNA NF₅₀ cut-off value based on Per-Protocol population.

TNA NF₅₀ = Toxin-neutralizing antibody 50% neutralization factor; TNA NF₅₀ LLOQ = 0.064. TNA NF₅₀ values below the lower limit of quantitation (LLOQ) were imputed as 0.032 in the calculations of Mean/SD/GMT, which was half of the LLOQ of the assay.

CI = Confidence interval

¹PP Population excludes site US1027.

²The 95% CI was based on the exact Clopper-Pearson confidence limit.

Ref: STN 125761/0, EBS.AVA.212, CSR Addendum 2, Table 9, page 25 of 73. Source: Table 14.2.4b

The LB of the two-sided 95% CI for the difference (AV7909 – BioThrax) in the percentage of subjects with TNA NF₅₀ values of ≥0.29 on Day 64 in the combined AV7909 group vs. BioThrax group was 25.2%, which is greater than the pre-defined criterion of -15%. Thus, the immune response at Day 64 in subjects who received AV7909 was determined as non-inferior to the immune response at Day 64 in subjects who received BioThrax. Negligible differences in the 95% CI of difference in percent (%) of AV7909-BioThrax were observed for data acquired from all study sites compared to data with site US1027 excluded (data not shown; STN 125761/0, EBS.AVA.212, CSR, Table 19, page 72 of 247. Source: Table 14.2.4).

Reviewer comment: *The BioThrax TNA responses observed in EBS.AVA.212 were lower-than-expected, based on TNA responses seen in the BLA application of BioThrax for PEP (STN 103821/5344). The Applicant was not able to identify a cause for this discrepancy—potency testing of the BioThrax lot did not indicate reduced potency. Irrespective of the lower-than-expected TNA responses for BioThrax, AV7909 met the TNA threshold of protection based on bridging of human immune responses to those seen in NHP and rabbit studies and met prespecified success criteria for demonstration of effectiveness.*

Success criteria for both co-primary immunogenicity endpoints were met by AV7909, demonstrating a protective level of immunity per the Animal Rule at Day 64, for all clinical study sites and when site US1027 was excluded.

6.1.11.2 Analyses of Secondary Endpoints

Secondary Immunogenicity Endpoint: AV7909 Immunogenicity at Day 29

One secondary immunogenicity endpoint was prespecified in EBS.AVA.212—AV7909 immunogenicity at Day 29; defined as the percentage of subjects in the combined AV7909 group (three lots pooled) with TNA NF₅₀ values ≥0.15 on Day 29.

Reviewer comment: *The Applicant considered this secondary immunogenicity endpoint clinically relevant because it would provide AV7909 protection data at an earlier time point post-vaccination (i.e., Day 29). Although not powered for this endpoint, immune response data at this early time point might be informative in a real-world post-exposure scenario where patients might not be 100% compliant with the full 60-day PEP antibiotic regimen.*

As shown in Table 9 below, there were 2437 subjects in the combined AV7909 group with TNA NF₅₀ of ≥0.15 at Day 29, corresponding to 97.8% who met the TNA threshold (95% CI: 97.2%,

98.4%). The pre-specified immunogenicity endpoint was met, as the LB of the two-sided 95% CI was 97.2%, which was greater than the pre-defined success criterion of $\geq 67\%$.

Table 9. Study EBS.AVA.212: Secondary Immunogenicity Endpoint: Percentage of Subjects with TNA NF₅₀ \geq 0.15 with AV7909 at Day 29 (PP Population¹)

Secondary Immunogenicity Endpoint: TNA NF ₅₀ \geq 0.15 at Day 29	AV7909 (Three Lots Pooled) (N=2670)
Number of subjects with TNA NF ₅₀ value at Day 29	2491
n, Percent (%) of subjects with TNA NF ₅₀ \geq 0.15 ²	2437 (97.8)
95% CI ^{3,4}	97.2, 98.4

N = Number of subjects per study group in the Per-Protocol population; n (%) = Number and percentage of subjects achieving a TNA NF₅₀ cut-off value based on the Per-Protocol population; TNA NF₅₀ = Toxin-neutralizing antibody 50% neutralization factor; TNA NF₅₀ LLOQ = 0.064. TNA NF₅₀ values below the lower limit of quantitation (LLOQ) were imputed as 0.032 in the calculations of Mean/SD/GMT, which was half of the LLOQ of the assay. CI = Confidence interval

¹PP Population excludes site US1027.

²Percentage was based on number of subjects with TNA NF₅₀ value at Day 29.

³The 95% CI was based on the exact Clopper-Pearson confidence limit.

⁴AV7909 was considered as having achieved the pre-specified immunogenicity criterion if the lower bound of the two-sided 95% CI of the percentage is $\geq 67\%$.

Ref: STN 125761/0, EBS.AVA.212, CSR Addendum 2, Table 10, page 26 of 73. Source: Table 14.2.5b

Reviewer comment: All prespecified primary and secondary immunogenicity endpoints were met in Study EBS.AVA.212. AV7909 demonstrated effectiveness, based on the prospectively defined immune criteria.

6.1.11.3 Subpopulation Analyses

Subgroup analyses of immunogenicity data were tabulated by age group, sex, and race. No formal statistical hypothesis testing was performed.

Subpopulation Analysis of Immunogenicity by Age

Evaluation of the consistency of immunogenicity results (TNA NF₅₀ on Days 29 and 64) by age for AV7909 (three lots pooled) and the BioThrax group were summarized by the following age groups: 18-30 years old, 31-50 years old, and 51-65 years old).

The immunogenicity response by age is provided in Table 10 below.

Table 10. Study EBS.AVA.212: Immunogenicity Response TNA NF₅₀ by Study Visit and Age Group (PP Population¹)

Parameter	AV7909 (Three Lots Pooled) 18-30 Years (N=800)	AV7909 (Three Lots Pooled) 31-50 Years (N=1105)	AV7909 (Three Lots Pooled) 51-65 Years (N=638)	BioThrax 18-30 Years (N=132)	BioThrax 31-50 Years (N=209)	BioThrax 51-65 Years (N=89)
Day 29	--	--	--	--	--	--
n	779	1081	631	132	207	88
GMT	2.011	1.407	1.146	0.354	0.201	0.174
95% CI	1.890, 2.139	1.322, 1.497	1.055, 1.244	0.290, 0.433	0.169, 0.238	0.130, 0.233
n, Percent (%) of subjects with TNA NF ₅₀ \geq 0.15	774 (99.4)	1057 (97.8)	606 (96.0)	NA	NA	NA
95% CI	98.5, 99.8	96.7, 98.6	94.2, 97.4	NA	NA	NA
Day 64	--	--	--	--	--	--
n	800	1105	638	132	209	89
GMT	0.942	0.683	0.629	0.402	0.298	0.311

Parameter	AV7909 (Three Lots Pooled) 18-30 Years (N=800)	AV7909 (Three Lots Pooled) 31-50 Years (N=1105)	AV7909 (Three Lots Pooled) 51-65 Years (N=638)	BioThrax 18-30 Years (N=132)	BioThrax 31-50 Years (N=209)	BioThrax 51-65 Years (N=89)
95% CI	0.891, 0.996	0.646, 0.721	0.584, 0.678	0.342, 0.473	0.259, 0.343	0.246, 0.392
n, Percent (%) of subjects with TNA NF ₅₀ ≥0.56	637 (79.6)	677 (61.3)	371 (58.2)	54 (40.9)	54 (25.8)	26 (29.2)
95% CI	76.7, 82.4	58.3, 64.2	54.2, 62.0	32.4, 49.8	20.0, 32.3	20.1, 39.8
n, Percent (%) of subjects with TNA NF ₅₀ ≥0.29	735 (91.9)	944 (85.4)	524 (82.1)	94 (71.2)	120 (57.4)	50 (56.2)
95% CI	89.8, 93.7	83.2, 87.5	78.9, 85.0	62.7, 78.8	50.4, 64.2	45.3, 66.7

¹PP Population excludes site US1027.

N = Number of subjects per study group in the PP Population; n = Number of subjects of subjects achieving a TNA NF₅₀ cut-off value based on the Per-Protocol population; % = Percentage was based on the number of subjects with non-missing TNA NF₅₀ in each group. GMT = Geometric mean titer; NA: Not available. TNA NF₅₀ = Toxin-neutralizing antibody 50% neutralization factor; TNA NF₅₀ values below the lower limit of quantitation (LLOQ) were imputed as 0.032 in the calculations of Mean/SD/GMT, which was half of the LLOQ of the assay. 95% CI was based on the exact Clopper-Pearson confidence limit.

Ref: STN 125761/0, EBS.AVA.212, CSR Addendum 2, Table 12, pages 28-29 of 73. Source: Table 14.2.7.1b.

The largest proportion of subjects by age comprising both the AV7909 and BioThrax groups were the 31 to 50-year age group. Immune responses for each of the AV7909 age groups were higher than those measured in the BioThrax age groups. Younger subjects (e.g., 18-30 years) in both the AV7909 and BioThrax age groups had higher GMTs and a greater percentage of subjects meeting protective TNA thresholds at both the Day 29 and 64 visits than older subjects (e.g., 31-50 years and 51-65 years). Age related findings were negligible when comparing TNA NF₅₀ levels observed for all study sites combined vs. levels observed with exclusion of study site US1027 immunogenicity data.

Reviewer comment: *The higher immune responses seen in younger study subjects is consistent with prior subpopulation study results described for BioThrax both for PrEP and PEP (under STNs 103821/5203 and 103821/5344, respectively). These results are expected due to immunosenescence, and generally lower post-vaccination antigen-specific antibody responses seen with aging.³⁶*

Subpopulation Analysis of Immunogenicity by Sex

Assessment of the immunogenicity response by the TNA NF₅₀ by study visit and sex indicated slightly lower GMTs on Day 29 in female than male subjects in the combined AV7909 group, though the percentage of subjects with a TNA NF₅₀ ≥0.15 was similar between females and males. For subjects vaccinated with BioThrax, GMTs were numerically lower in males than females. The TNA NF₅₀ was not measured in BioThrax subjects, therefore this information was not available for comparison.

On Day 64, there was no meaningful between-sex difference observed for immunogenicity response (GMTs and TNA NF₅₀ ≥0.56, n (%)) in the combined AV7909 group, in contrast to the BioThrax group, where the immunogenicity response was higher in females compared with males.

On Day 64, the GMT TNA NF₅₀ was higher in the combined AV7909 group compared with the BioThrax group for both females (0.746 versus 0.404) and males (0.729 versus 0.268). Similarly, the percentage of subjects who achieved a TNA NF₅₀ ≥0.56 at Day 64 was higher in the combined AV7909 group compared with the BioThrax group for both females (67.5% versus 39.7%) and males (64.8% versus 23.2%).

Reviewer comment: *While immune responses were generally observed to be similar between females and males vaccinated with AV7909, they were generally lower in males than females in BioThrax vaccinated subjects. Immune responses to BioThrax have been previously described as higher in females than males (STN 103821/5023). Immune responses in the combined AV7909 groups were numerically higher at both Days 29 and 64, when compared to BioThrax.*

Subpopulation Analysis of Immunogenicity by Race

Assessment of the immunogenicity response (TNA NF₅₀) by study visit and race indicated a generally higher response in the combined AV7909 group compared with the BioThrax group, although the number of subjects in some race groups (i.e., Other/More than One Race) was too small to derive conclusive results. In general, no clinically meaningful difference in the immune response by race was observed at Days 29 and 64 for either the combined AV7909 group or the BioThrax group.

Reviewer comment: *There was no significant difference in the immune response in AV7909 vaccinated subjects when evaluated by racial subgroup (White vs. Black/African American vs. Other/More than One Race).*

6.1.11.4 Dropouts and/or Discontinuations

Randomized subjects who withdrew from the study for any reason were not replaced. Subjects who withdrew from the study but received at least one dose of vaccination were encouraged to comply with 'safety-only' follow up procedures for the remainder of the study duration through Month 13. Reasons for withdrawal of individual subjects from the study prior to the Month 13 final safety follow-up phone call were to be recorded on the eCRF. Missing data were not imputed.

Reviewer comment: *The Applicant's handling of subject withdrawals and missing data was appropriate.*

6.1.11.5 Exploratory and Post Hoc Analyses

Predicted Vaccine Efficacy (PVE) at Days 64 and 29 were prespecified exploratory endpoints. The PVE and associated 95% CI were calculated at Day 64 and Day 29 for the combined AV7909 groups using animal data from Emergent's NHP Good Laboratory Practice (GLP) Study 3655-100072763 and the observed TNA NF₅₀ values from Study EBS.AVA.212. The computational algorithm with double-bootstrap method to calculate CIs proposed by Kohberger³⁷ (Kohberger, 2007) was used (details provided in Appendix II of the SAP [STN 125761/0, EBS.AVA.212, CSR, Appendix 16.1.9]).

The PVE for AV7909 is summarized in Table 11 below.

Table 11. Study EBS.AVA.212: Exploratory Endpoint: AV7909 Predicted Vaccine Efficacy (PVE) at Study Day 29 and 64 (PP Population¹)

Human Time Point	Study 3655 NHP Model Time Point	Study 3655 NHP Model NF ₅₀ Threshold ²	AV7909 (Three Lots Pooled) N	AV7909 (Three Lots Pooled) NF ₅₀	AV7909 (Three Lots Pooled) Number and Percentage Meeting Threshold (95% CI) ³	AV7909 (Three Lots Pooled) Predicted VE (95% CI) % ⁴
Day 29	Day 28	0.15	2491	1.493	2437, 97.8 (97.2, 98.4)	96.8 (92.4, 98.9)
Day 64	Day 70	0.26	2543	0.740	2256, 88.7 (87.4, 89.9)	82.9 (55.1, 96.7)

TNA = Toxin-neutralizing antibody. NF₅₀ = 50% neutralization factor. LLOQ = Lower limit of quantitation; TNA NF₅₀ values below the LLOQ were imputed as 0.032, which was half of the LLOQ of the assay.

VE = Vaccine Efficacy; CI = Confidence interval; GLP = Good Laboratory Practice.

¹PP Population excludes site US1027.

²A logistic regression model with log-transformed NF₅₀ values as the predictor and survival status as the response was used to derive the NF₅₀ threshold associated with 70% probability of survival on Study Days 28 and 70 in Emergent's non-human primate (NHP) GLP Study 3655-100072763. The thresholds were presented in Table 13 and 15 in the Study 3655-100072763 final study report.

³95% CI was based on the exact Clopper-Pearson confidence limit.

⁴AV7909 PVE and associated 95% CI were calculated at Day 29 and Day 64 using animal data from Emergent's NHP GLP Study 3655-100072763 and the observed TNA NF₅₀ values in clinical study EBS.AVA.212. The computational algorithm with double-bootstrap method (1000 times of re-samplings in animal and human data) proposed by Kohberger (Kohberger, 2007)³⁷ was implemented to calculate the PVE and the 95% CIs.

Ref: STN 125761/0, EBS.AVA.212, CSR Addendum 2, Table 11, page 27 of 73. Source: Table 14.2.6b

In the combined AV7909 group, the PVE for AV7909 was 96.7% (95% CI: 92.4%, 98.8%) at Day 29 and 82.9% (95% CI: 55.0%, 96.8%) at Day 64.

Reviewer comment: *The PVE is not a true estimate of VE but rather a number extrapolated from NHP challenge studies and the observed TNA NF₅₀ values from Study EBS.AVA.212.*

6.1.12 Safety Analyses

6.1.12.1 Methods

Safety monitoring in EBS.AVA.212 was previously summarized in Section 6.1.7

'Surveillance/Monitoring.' Safety analyses were performed on the Safety Population, defined as all randomized subjects who received at least one vaccination. Safety analyses were conducted with revised safety data that excluded site US1027 (STN 125761/0/27 and 125761/0/42) to rectify GCP issues identified this study site and dataset discrepancies and inconsistencies identified during BLA review.

Reviewer comment: *Safety datasets used for review of EBS.AVA.212 comprise revised datasets submitted under BLA STN 125761/0/27 and CSR Addendum 3 along with corrected reactogenicity and TEAE tables submitted under 125761/0/38. Updates to the safety datasets resulted in no changes to abnormal clinical laboratory (hematology, serum chemistry, urinalysis) AEs, AEs stemming from PE or VS findings, SAEs, AESIs, or pregnancy/fetal outcomes, but did result in changes to AE reactogenicity data recorded on e-diary cards and slight changes to the TEAE frequencies (see discussion of e-diary solicited AE and unsolicited TEAEs) for Study EBS.AVA.212.*

6.1.12.2 Overview of Adverse Events

Extent of Exposure

Exposure to study vaccinations was previously summarized in Section 6.1.10.1.3 ‘Subject Disposition’ in the clinical review.

Solicited Adverse Reactions

Solicited AEs comprising local and systemic reactogenicity signs and symptoms were assessed by subjects via e-diary and by investigators, through in-clinic assessment.

Solicited Injection Site and Systemic Reactions (E-Diary Card Evaluation)

Subjects completed a web-based subject diary for seven days after each vaccination starting on Day 1. Data on injection site reactions and systemic reactions were solicited.

Most subjects in the combined AV7909 group (2460/3151, 78.1%) who received at least one vaccination were compliant with the e-diary completion (defined as >75% completion of the e-diary for 7 days). The proportion of subjects who were compliant with e-diary completion was higher following the first (2435/3151, 77.3%) and second (2160/2898, 74.5%) vaccinations in comparison to the third vaccination (1715/2733, 62.8%) (125761/0/38, Table 14.3.4.9b, E-diary Compliance, page 1). The same trend was observed in the BioThrax group (e-diary compliance range: 69.7%-81.1%) (125761/0/38, Table 14.3.4.9b, E-diary Compliance, page 1).

Local Reactogenicity as Captured by E-Diary Card

A summary of the frequency of e-diary recorded injection site reactions of all severity grades and injection site reactions ≥Grade 3 severity after the first two vaccinations of AV7909 (AV7909 groups received matching saline placebo for their third vaccination) compared to BioThrax is provided in Table 12 below.

Table 12. Study EBS.AVA.212: Number and Percentage of Subjects with Injection Site Reactions on Electronic Diary (E-Diary) Cards after Each Vaccination with AV7909 (All Lots Pooled) compared to BioThrax (Safety Population¹)

Injection Site Reaction	AV7909 1st Vaccination N=3151 n (%)²	BioThrax 1st Vaccination N=533 n (%)²	AV7909 2nd Vaccination N=2898 n (%)²	BioThrax 2nd Vaccination N=493 N (%)²
Any injection site reaction	--	--	--	--
Any grade	2739 (86.9)	485 (91.0)	2426 (83.7)	412 (83.6)
≥ Grade 3	59 (1.9)	7 (1.3)	63 (2.2)	12 (2.4)
Tenderness	--	--	--	--
Any grade	2503 (81.1)	450 (85.9)	2262 (80.5)	385 (80.0)
≥ Grade 3	30 (1.0)	2 (0.4)	24 (0.9)	2 (0.4)
Pain	--	--	--	--
Any grade	2392 (75.9)	426 (79.9)	2179 (75.2)	367 (74.4)
≥ Grade 3	41 (1.3)	3 (0.6)	27 (0.9)	2 (0.4)
AML ³	--	--	--	--
Any grade	1531 (48.6)	210 (39.4)	1477 (51.0)	166 (33.7)
≥ Grade 3	29 (0.9)	0	25 (0.9)	2 (0.4)
Warmth	--	--	--	--
Any grade	1087 (35.2)	247 (47.1)	1082 (38.5)	263 (54.7)
≥ Grade 3	12 (0.4)	24 (4.6)	10 (0.4)	0
Induration	--	--	--	--
Any grade	780 (24.8)	303 (56.8)	759 (26.2)	299 (60.6)
≥ Grade 3	6 (0.2)	3 (0.6)	3 (0.1)	3 (0.6)

Injection Site Reaction	AV7909 1 st Vaccination N=3151 n (%) ²	BioThrax 1 st Vaccination N=533 n (%) ²	AV7909 2 nd Vaccination N=2898 n (%) ²	BioThrax 2 nd Vaccination N=493 N (%) ²
Bruise	--	--	--	--
Any grade	321 (10.2)	107 (20.1)	274 (9.5)	106 (21.5)
≥ Grade 3	5 (0.2)	0	3 (0.1)	0
Itching	--	--	--	--
Any grade	319 (10.1)	109 (20.5)	472 (16.3)	236 (47.9)
≥ Grade 3	7 (0.2)	0	6 (0.2)	2 (0.4)
Swelling	--	--	--	--
Any grade	314 (10.0)	167 (31.3)	446 (15.4)	214 (43.4)
≥ Grade 3	4 (0.1)	1 (0.2)	7 (0.2)	5 (1.0)
Erythema/ Redness	--	--	--	--
Any grade	227 (7.2)	170 (31.9)	417 (14.4)	210 (42.6)
≥ Grade 3	7 (0.2)	1 (0.2)	20 (0.7)	4 (0.8)

N = Number of subjects in the safety population who received the first or second vaccination.

n = Number of subjects with a reaction. % = n/N*100 (reflects subjects who did not have missing e-diary data).

¹Safety Population excludes site US1027.

²For each vaccination site, each subject was counted only once under the most severe intensity rating.

³AML: Arm motion limitation.

Ref: STN 125761/0/31, EBS.AVA.212, CSR Addendum 3, Table 14.3.4.10.1c, Pages 1-5. Source: Listing 16.2.8.9.1; STN 125761/0/38, EBS.AVA.212, CSR Addendum 3, Table 14.3.4.10.1b, Pages 1-5. Source: Listing 16.2.8.9.1.

Injection site reactions were common for both the AV7909 and BioThrax groups post-vaccination and were most frequent after the first vaccination. The majority of local reactogenicity signs or symptoms were Grade 1. Grade 3 local reactions were infrequent for each respective injection site sign or symptom ($\leq 1\%$ frequency per local solicited symptom or frequency $\leq 2.3\%$ total for all reactogenicity symptoms reported) for both AV7909 and BioThrax vaccinated subjects.

The most common injection site reactions reported by e-diary (in order of frequency) for both AV7909 and BioThrax were injection site tenderness and pain. Induration, warmth, swelling, erythema/redness, itching, bruise, and swelling were more commonly reported after BioThrax vaccination than with AV7909. AML was slightly higher in frequency in subjects vaccinated with AV7909 than with BioThrax.

Reviewer comment: *While the frequency of tenderness and pain post-vaccination were relatively comparable between AV7909 and BioThrax, subjects vaccinated with BioThrax generally reported more frequent signs and symptoms associated with injection site reactions than those vaccinated with AV7909. Local reactogenicity reported in AV7909 subjects after the third vaccination was significantly lower (data not shown; STN 125761/0/31, EBS.AVA.212, CSR Addendum 3, Table 14.3.4.10.1b, Pages 1-5; Source: Listing 16.2.8.9.) than reported for the prior two vaccinations because AV7909 subjects received matching placebo as their third vaccination.*

Exclusion of e-diary card local reactogenicity data from site US1027 using revised datasets generally changed frequencies of select local reactogenicity events for subjects vaccinated with AV7909 and BioThrax, as summarized below, with minimal numerical and frequency changes in all other local reactogenicity events:

Key changes in local reactogenicity frequency in the revised datasets submitted under EBS.AVA.212 CSR Addendum 3 are the following:

- AV7909 group:

- Decrease in the frequency of erythema/redness from 314 events (10.0%) after the first vaccination to 227 events (7.2%), and
- Increase in the frequency of bruise after the first vaccination from 226 events (7.2%) to; 321 events (10.2%). Decrease in the frequency of bruise after the second vaccination from 418 events (14.4%) to 274 events (9.5%).
- BioThrax group:
 - Increase in Grade 3 warmth from 0 events (0%) after the first vaccination to 24 events of Grade 3 warmth (4.5%).

In summary, the reanalyzed local reactogenicity data for Study EBS.AVA.212 did not significantly change the reactogenicity results and conclusions regarding local reactogenicity assessed by e-diary card; though for a few select injection site symptoms (see above), a small numerical difference in the reactogenicity rate for the respective sign/symptom was observed after data reanalysis.

Systemic Reactogenicity as Captured by E-Diary Card

The frequency of post-vaccination systemic reactions of all severity grades and systemic reactions ≥Grade 3 severity recorded on subject e-diary cards, is summarized in Table 13 below.

Table 13. Study EBS.AVA.212: Number and Percentage of Subjects with Systemic Reactions on Electronic Diary (E-Diary) Cards after Each AV7909 Vaccination (All Lots Pooled) compared to BioThrax (Safety Population¹)

Injection Site Reaction	AV7909 1 st Vaccination N=3151 n (%) ²	BioThrax 1 st Vaccination N=533 n (%) ²	AV7909 2 nd Vaccination N=2898 n (%) ²	BioThrax 2 nd Vaccination N=493 n (%) ²
Any systemic reaction	--	--	--	--
Any grade	2334 (74.1)	362 (67.9)	2028 (70.0)	285 (57.8)
≥ Grade 3	109 (3.5)	7 (1.3)	104 (3.6)	9 (1.8)
Muscle ache				
Any grade	1991 (63.2)	286 (53.7)	1702 (58.7)	195 (39.6)
≥ Grade 3	61 (1.9)	5 (0.9)	47 (1.6)	4 (0.8)
Fatigue/tiredness	--	--	--	--
Any grade	1626 (51.6)	213 (40.0)	1500 (51.8)	180 (36.5)
≥ Grade 3	48 (1.5)	3 (0.6)	46 (1.6)	4 (0.8)
Headache	--	--	--	--
Any grade	1281 (40.7)	180 (33.8)	1176 (40.6)	138 (28.0)
≥ Grade 3	47 (1.5)	4 (0.8)	53 (1.8)	5 (1.0)
Fever ³	--	--	--	--
Any grade	82 (2.6)	1 (0.2)	142 (4.9)	4 (0.8)
≥ Grade 3	5 (0.2)	0	14 (0.5)	1 (0.2)

N = Number of subjects in the safety population who received the first, second, third or any vaccination; n = Number of subjects with a reaction. % = n/N*100 (reflects subjects who did not have missing e-diary data); Rxn: Reaction

¹Safety Population excludes site US1027.

²For each vaccination site, each subject was counted only once under the most severe intensity rating.

³The toxicity grade for fever is based on the combination of oral temperature subjects self-reported in the e-diary and any additional oral temperature readings provided by the subject. Toxicity grades were set to NULL for temperature less than 90 F or greater than 110 F, except for two records with Grade 3 reported as Celsius and confirmed by PI.

Ref: STN 125761/0/31, EBS.AVA.212, CSR Addendum 3, Table 14.3.4.10.2c, pages 1-3. Source: Listing 16.2.8.9.3; STN 125761/0/38, EBS.AVA.212, CSR Addendum 3, Table 14.3.4.10.2b, pages 1-3.

Systemic reactions were slightly higher in frequency and severity in subjects vaccinated with AV7909 over BioThrax. Overall, systemic reactions were reported quite frequently for both treatment arms. Unlike local reactogenicity, the frequency of systemic reactions did not

significantly differ numerically between the first and second vaccinations. The most common systemic reactions reported in both AV7909 and BioThrax vaccinated subjects comprised (in order of frequency) muscle ache (myalgia), fatigue/tiredness, and headache. Reports of post-vaccination fever were low for both AV7909 and BioThrax, though numerically slightly higher in frequency in AV7909-vaccinated subjects. Reanalysis of systemic reactogenicity by e-diary card using the revised safety datasets and excluding site US1027 (EBA.AVA.212, CSR Addendum 3) indicated minimal changes in the number and frequency of systemic reactogenicity events and showed no change in the systemic reactogenicity profile of AV7909 compared with BioThrax or conclusions regarding systemic reactogenicity.

Reviewer comment: *In summary, local reactogenicity appeared to be more frequent and severe in BioThrax vaccinated subjects—most likely because BioThrax was administered as 3, SC doses in comparison to AV7909 given as 2, IM doses, while systemic reactions were slightly more frequent and severe in AV7909 vaccinated subjects. A plausible mechanistic explanation for the greater systemic reactogenicity in AV7909 vaccinated subjects may be due to the addition of CpG 7909 and its effects on immune stimulation, particularly proinflammatory effects due to activation of Th1-mediated immune responses.^{28,29}*

Solicited Injection Site and Systemic Reactions (In-Clinic Evaluation)

Local and systemic reactogenicity was assessed in-clinic by the investigator or designee 30 minutes after administration of each dose of IP.

In-Clinic Local Reactogenicity

Local reactogenicity results for AV7909 and BioThrax vaccinated subjects at each clinic visit (Visits 1 and 2) indicate that the incidence of in-clinic injection site reactions was infrequent for both the AV7909 and BioThrax groups post-vaccination; with most subjects reporting no injection site reactions (i.e., Grade 0) after any vaccination (2671/3151 [84.8%] subjects in the combined AV7909 group and 436/533 [81.8%] subjects in the BioThrax group). There was no meaningful difference in the proportion of subjects with any injection site reactions collected in-clinic after the first, the second, the third, or after 'any vaccination' for the combined AV7909 group or the BioThrax group (data not shown; STN 125761/0, EBS.AVA.212, CSR Addendum 2 Table 24, page 50 of 73; data not shown; BLA STN 125761/0/27, EVS.AVA.212, CSR Addendum 3, Table 13, page 35 of 57).

Reviewer comment: *Reanalysis of in-clinic local reactogenicity data with the revised datasets that excluded site US1027 indicated no change in the number or frequency of post-vaccination injection site reactions for all local reactogenicity signs and symptoms at each in-clinic visit (data not shown; Ref: STN 125761/0, EBS.AVA.212 CSR Addendum 2, 14.3.4.13.1b, Pages 1-5; STN 125761/0/27, EBS.AVA.212 CSR Addendum 3, Table 14.3.4.13.1b, pages 1-5. Source: Listing 16.2.8.8.1).*

In-Clinic Systemic Reactogenicity

Systemic reactogenicity results for AV7909 and BioThrax vaccinated subjects at each clinic visit (Visits 1 and 2) indicate that the incidence of systemic reactions in-clinic was infrequent for both the AV7909 and BioThrax groups post-vaccination; with most subjects reporting no systemic reactions (i.e., Grade 0) after any vaccination. There were no reports of fever; apart from of one subject with Grade 1 fever reported 30 minutes after the first vaccination with AV7909 (STN 125761/0/27, EBS.AVA.212, CSR). The majority of reported systemic reactions were Grade 1 in severity. There were no reported Grade 3 systemic reactions.

Reviewer comment: *Local and systemic reactions observed in-clinic were markedly lower in frequency than reported on e-diary—most likely because signs and symptoms were assessed 30 minutes post-vaccination and not observed over longer time periods, which would have more accurately captured the temporal evolution of injection site and systemic reactions. There was no significant difference observed in the frequency or severity of local and systemic reactions between AV7909 and BioThrax subjects in-clinic.*

Demographic Subgroup Analysis of Injection Site and Systemic Reactions (E-Diary)

By Age:

Injection site reactions (local reactogenicity), as reported by e-diary, were generally more frequent and greater in severity in younger subjects (18-30 years; n=992) vaccinated with AV7909 than older subjects (31-50 years; n=1380 and 51-65 years; n=779). A somewhat higher proportion of subjects (5-10%) 31-50 and 51-65 years of age reported Grade 1 local reactions, whereas a somewhat higher proportion of younger subjects (18-30 years) reported Grade 2 local reactions. This was seen after each vaccination and for all vaccinations combined (data not shown; STN125761/0/38, Table 14.3.4.11.1.b, pages 1-20). A similar trend of greater local reactogenicity in younger subjects was reported with BioThrax. In contrast, induration and bruising was more frequently reported in older subjects (51-65 years), with a higher incidence after the second and third vaccinations. Systemic reactions, especially fatigue (tiredness) and fever were reported with slightly higher incidence in younger subjects (18-30 years) than in older subjects who received AV7909 (or BioThrax, data not shown; BLA STN125761/0/38, Table 14.3.4.12.1b, pages 1-12).

By Sex:

Female subjects (n=1826) vaccinated with AV7909 reported a slightly higher incidence of local injection site reactions by e-diary, which were slightly higher in severity rating (more Grade 2 and 3 reactions reported) than male (n=1325) subjects (data not shown; STN 125761/0/38, Table 14.3.4.11.26, pages 1-20). A similar trend was reported in subjects vaccinated with BioThrax.

Systemic reactions were also reported in e-diaries with a higher incidence and somewhat higher severity in females than males who received AV7909 or BioThrax (data not shown; STN125761/0/38, Table 14.3.4.12.2b, pages 1-12).

By Race:

There were no consistent trends seen with injection site reactions reported post-vaccination with AV7909 (or BioThrax) by e-diary across all symptoms assessed, based on race. A slightly higher proportion of White subjects (n=2452) reported tenderness overall (all vaccinations combined) than Black/African American subjects (n=541) or subjects in the 'Other/More than One Race' subgroup (n=158) (data not shown; STN125761/0/38, Table 14.3.4.11.3b, pages 1-20). Bruising, induration, erythema/redness, and swelling were reported in a somewhat higher proportion of Black/African American subjects for all vaccinations combined than in the other racial subgroups. Pain and AML were reported in a higher proportion of subjects in the 'Other' racial subgroup. Because of the relatively small number of subjects in the 'Other' category, it is difficult to make generalized comments about this racial subgroup and reported local reactogenicity. There were no consistent trends seen after the first, second, or third vaccination.

Similarly, no consistent trends were reported for systemic reactions reported by e-diary, though a slightly higher proportion of subjects in the 'Other' subgroup reported headache and muscle

ache (all vaccinations combined) (data not shown; STN125761/0/38, Table 14.3.4.12.3b, pages 1-12).

Summary of Demographic Subgroup Analysis of Local and Systemic Reactogenicity Assessed by E-diary

A slightly greater proportion of injection site reactions (local and systemic reactogenicity), with a slightly higher prevalence of more severe reactions (Grade 2 or 3) after AV7909 administration were seen in the youngest subgroup of subjects (18-30 years of age) and in female subjects. The results for Study EBS.AVA.212 are consistent with subgroup reactogenicity findings that were also observed for BioThrax under STN 103821/5203. Racial differences in local and systemic reactogenicity after AV7909 vaccination were inconsistent across all symptoms evaluated such that no racial subgroup appeared to have a consistently higher prevalence or severity of reactogenicity symptoms. No trend in local or systemic reactogenicity was consistently observed related to AV7909 vaccination dose number when assessed by racial groups.

Treatment Emergent Adverse Events (TEAEs)

The most frequent TEAEs reported in the combined AV7909 group were generally attributable to post-vaccination injection site reactions and comprised the following (in order of decreasing frequency): injection site pain (AV7909: 4.6%; BioThrax: 9.2%), vaccination complication (AV7909: 3.6%; BioThrax: 4.7%), musculoskeletal procedural complication (AV7909: 2.9%; BioThrax: 3.6%), upper respiratory tract infection (AV7909: 2.9%; BioThrax: 2.3%), 'procedural' or post-vaccination headache (AV7909: 2.8%; BioThrax: 4.9%), and injection site induration (AV7909: 2.2%; BioThrax: 2.9%).

The overall incidence of the most common TEAEs ($\geq 1\%$ in any treatment group) was numerically lower in the combined AV7909 group (530/3151 subjects, 16.8%) compared with the BioThrax group (167/533 subjects, 31.3%). Excluding injection site pain and vaccination complications, all TEAEs occurred in $\leq 3\%$ subjects within the combined AV7909 group. TEAEs of nausea, upper respiratory infection, urinary tract infection, back pain and headache were reported at a slightly higher frequency in AV7909 vaccinated subjects than in those who received BioThrax.

The number and frequency of most TEAEs increased slightly in AV7909 vaccinated subjects when TEAE reports were reanalyzed using the revised datasets provided under STN 125671/0/27 and 125761/0/31, with the exception of TEAEs occurring in at least 1% of all subjects for the BioThrax group which increased significantly from 103 subjects (19.3%) to 167 subjects (31.3%) using the revised safety datasets (STN 125761/0, EBS.AVA.212, CSR Addendum 2, Table 17, page 37 of 73; Source: Tables 14.3.1.1b and 14.3.1.7b) (31.3%; see Table 12).

Reviewer comment: *The slight increase in TEAEs reported in AV7909 vaccinated subjects in the revised datasets generally reflects the slightly increased frequency of injection site pain, injection site induration, injection bruising, injection site pruritus, and vaccination complication in these subjects.*

The majority of TEAEs (all TEAEs and IP-related TEAEs) reported were either Grade 1 or Grade 2 in severity. Grade 3 and Grade 4 AEs were less frequent and occurred in slightly higher proportions in the combined AV7909 group (179/3151 [5.7%] and 21/3151 [0.7%] subjects, respectively) compared to the BioThrax group (6/533 [4.9%] and 3/533 [0.6%] subjects, respectively) (STN 125761/0/27, EBS.AVA.212, CSR Addendum 3, Table 8, page 22 of 57).

To evaluate the consistency of the safety findings across subgroups, the incidence of TEAEs were summarized for AV7909 (three lots pooled) and BioThrax groups by age (18-30 years old, 31-50 years old, and 51-65 years old), sex (male, female), and race (White, Black/African American, Other/More than One Race). No formal statistical hypothesis testing was performed.

Safety findings in the subgroup categories were consistent with the results in the overall Safety Population. Although the study was not powered to detect treatment differences between subgroups, the safety profile of AV7909 was generally consistent across age, sex, and racial subgroups (data not shown; STN 125761/0, EBS.AVA.212, CSR Addendum 2, Tables 14.3.1.9.2b, 14.3.1.5.3b and 14.3.1.5.4b, respectively; and STN 125761/0/27, EBS.AVA.212, CSR Addendum 3, page 24 of 57) with one exception. A greater proportion of female subjects reported TEAEs and IP-related TEAEs than male subjects in the combined AV7909 group (42.3% and 16.2% vs. 32.6% and 11.1%, respectively) and in the BioThrax group (52.9% and 38.0% vs. 38.4% and 22.0%, respectively); the majority of these TEAEs were related to post-vaccination reactogenicity. Similar trends were observed in the demographic subgroups excluding site US1027 data.

Reviewer comment: *Reanalysis of the TEAE data using the revised datasets and excluding site US1027 (per STN 125761/0/27) showed a numerically higher incidence of injection site reactions in BioThrax-treated subjects than in those who received AV7909. With the revised datasets, a slightly higher proportion of subjects in the combined AV7909 group reported more severe TEAEs (i.e., ≥Grade 3) than subjects vaccinated with BioThrax. The revised datasets did not appreciably change subgroup analysis of safety data and did not change the overall conclusions regarding safety of AV7909 related to the incidence of TEAEs across different demographic subgroups.*

Safety Findings from Serial Physical Exams Conducted during the Study

No significant findings or changes in the physical exam were reported throughout the study duration in either treatment group, aside from injection site reactions reported (see discussion above for Reactogenicity Findings and TEAEs).

6.1.12.3 Deaths

Six deaths were reported in EBS.AVA.212 (all in AV7909 vaccinated subjects); 5 out of the 6 deaths were a result of suicide or drug overdose/toxicity, as summarized in Table 14 below. The sixth subject who died, Subject US(b) (6), was a 30-year-old Black female who was found dead in someone’s house approximately 8.5 months after administration of the last dose of IP. The cause of death was unknown. All six subjects received the 3 doses of IP (2 doses of AV7909, followed by matching placebo). None were deemed related to IP by the investigator or Applicant (STN 125761/0, EBS.AVA.212, CSR Addendum, Section 14.3.3.1 Narrative of Deaths).

Table 14. EBS.AVA.212: Summary of all Deaths Reported (ITT Population)

Study	Subject Number	Treatment Group / Test Product	Age (years)/ Sex/Race ¹	Interval Between Last Vaccine Dose and Event	Preferred Term (MedDRA v22.0)	Causality
EBS.AVA.212	US(b) (6)	Group 1 / AV7909	52/F/W	Approximately 2 months	Toxicity to various agents (heroin)	Unrelated
EBS.AVA.212	US(b) (6)	Group 1 / AV7909	30/F/B	Approximately 8.5 months	Death (of unknown cause) ²	Unrelated

Study	Subject Number	Treatment Group / Test Product	Age (years)/ Sex/Race ¹	Interval Between Last Vaccine Dose and Event	Preferred Term (MedDRA v22.0)	Causality
EBS.AVA.212	US(b) (6)	Group 2 / AV7909	25/M/W	Approximately 2 months	Completed suicide	Unrelated
EBS.AVA.212	US(b) (6)	Group 2 / AV7909	24/M/O	78 Days	Completed suicide	Unrelated
EBS.AVA.212	US(b) (6)	Group 3 / AV7909	55/M/W	240 Days (8 months)	Overdose	Unrelated
EBS.AVA.212	US(b) (6)	Group 3 / AV7909	49/M/W	Approximately 8.5 months	Toxicity to various agents ⁴	Unrelated

EBS.AVA.212, Group 1 = AV7909 Lot 1; Group 2 = AV7909 Lot 2; Group 3 = AV7909 Lot 3.

¹Sex: M = Male, F = Female; Race: W = White, B = Black or African American, O = Other.

²Cause of death in Subject US(b) (6) : subject found dead in someone's house; cause of death unknown.

³Cause of death in Subject US(b) (6) : Grade 5 Kratom (mitragynine) toxicity resulting in an acute myocardial infarction.

⁴Mitragynine is a primary alkaloid derived from the tropical tree of the same name. It is purported to have psychoactive effects and is commonly known as Kratom to recreational users.

Ref: STN 125761/0, EBS.AVA.212, CSR Addendum, Section 14.3.3.1 Narrative of Deaths; Table 14.3.2.1.

Reviewer comment: *The frequency of deaths reported in EBS.AVA.212 was low; with none of the deaths reported 'related to' AV7909 administration.*

Apart from the six deaths reported in AV7909-vaccinated subjects, three female subjects who became pregnant while enrolled in study EBS.AVA.212 had an outcome of fetal death. Please see a discussion of fetal deaths under Section 9.1.1. Human Reproduction and Pregnancy Data.

6.1.12.4 Nonfatal Serious Adverse Events

Serious Adverse Events (SAEs)

SAEs were reported in 58 subjects who received AV7909 (58/3151, 1.8%) and four subjects who received BioThrax (4/533, 0.8%).

None of the SAEs by PT occurred in more than 0.2% of subjects within the combined AV7909 group or the BioThrax group.

There were two subjects who had SAEs that were considered 'possibly related' to IP by the investigator, but both SAEs were found to have underlying confounding factors that could account for the reported event:

1. AV7909 (Lot 1): SAE report of acute cholecystitis in Subject US(b) (6).
2. BioThrax: SAE report of sialadenitis and lymphadenitis in Subject US(b) (6).

Subject US(b) (6) was a 42-year-old White female who developed abdominal pain one day after and was hospitalized for fever and worsening abdominal pain 5 days after receipt of the second dose of AV7909. The subject underwent a laparoscopic cholecystectomy the same day (5 days post-receipt of the second vaccine dose) and pathological findings of the gallbladder revealed benign gallbladder with acute and chronic cholecystitis; cholelithiasis with multiple bile pigment type gallstones; and calculi obstruction of the lumen of the gallbladder body and fundus. The event of acute cholecystitis acute resolved 7-days after receipt of the second vaccine dose (Study Day 35) and the subject was discharged from the hospital. The subject completed the study.

The investigator assessed the event of Grade 4 acute cholecystitis as 'possibly related' to IP due to the short latency. The MM assessed the event to be 'not related' to IP administration, as

the anatomic pathology report showed a gross diagnosis of benign gallbladder showing acute and chronic cholecystitis and cholelithiasis, with multiple bile pigment type gallstones. The Applicant assessed the event of acute cholecystitis as 'not related' to AV7909, as the pathology report was consistent with the known slow development of gallstones.

Reviewer comment: *The clinical reviewer agrees with the Applicant's attribution of causality of this SAE as 'not related' to treatment, since the subject had pre-existing gallstone disease, with evidence of multiple gallstones that preceded vaccination.*

Subject US(b) (6) was a 22-year-old White female who developed lymphadenitis, sialadenitis, and streptococcal pharyngitis one day after the first and only dose of BioThrax. One week after vaccination, the subject was admitted to the hospital due to worsening symptoms. A computed tomography (CT) scan showed symmetric edema of the parotid and submandibular glands with adjacent submandibular free fluid and SC edema compatible with acute sialadenitis, symmetric enlargement of the adenoids, lingual tonsils, and palatine tonsils compatible with acute tonsillitis. Adjacent peritonsillar free fluid in the parapharyngeal and retropharyngeal spaces without loculated parapharyngeal or retropharyngeal fluid collection and reactive jugular chain lymphadenopathy was noted. Laboratory evaluation showed white blood cells (WBCs) at 15,75 K/ μ L (normal range: 3.99 to 11.19), neutrophils at 75.4% and segments and bands absolute at 11.85 (normal range: 1.64 to 7.28).

The subject required a 10-day treatment with antimicrobial therapy, along with steroids and non-steroidal drugs. The subject did not receive a second dose of vaccine due to this SAE but completed safety follow-up in the study. The event of streptococcal pharyngitis was resolved 13 days post-vaccination (Study Day 13). The events of lymphadenitis and sialadenitis were resolved 14 days post-vaccination (Study Day 14).

The investigator assessed the events of Grade 4 lymphadenitis, Grade 4 sialadenitis, and Grade 3 streptococcal pharyngitis as 'possibly related' to IP administration. The Applicant assessed the SAE of sialadenitis and lymphadenitis as 'not related' to IP because of co-existing Grade 3 streptococcal pharyngitis and because there was no biologic relationship noted between AV7909 administration and the bacterial infection. Streptococcal infection was considered a plausible explanation for the subject's sialadenitis and lymphadenitis.

Reviewer comment: *Given the preceding history of streptococcal throat infection prior to development of sialadenitis in Subject US(b) (6), the clinical reviewer agrees with the Applicant's categorization of this SAE as 'not related' to treatment, since this is the most plausible explanation for this event. It is possible that after administration of AV7909, the pro-inflammatory (Th1) response may have accentuated the subject's immune response in the setting of an ongoing infection with exacerbation of inflammation in adjacent organs (e.g., tonsils, adenoids, and lymph nodes); however, it is difficult to attribute this event to AV7909 alone, particularly the CpG 7909 component of the vaccine, since there was already a pre-existing infection.*

The two reported SAEs (n=1 in the AV7909 group and n=1 in the BioThrax group) were not impacted by the revised safety datasets or exclusion of study site US1027 (STN125671/0/27, EBS.AVA.212 CSR Addendum 3). Revision of the datasets did not change the SAE incidence in Study EBS.AVA.212.

Seven SAEs were reported in EBS.AVA.212 in seven pregnant subjects vaccinated with AV7909, with six of these comprising spontaneous abortion and the other a report of preterm

premature rupture of membranes (PPROM), that resulted in fetal death. One SAE of spontaneous abortion was reported in one pregnant female vaccinated with BioThrax (Subject US(b) (6) , see Section 9.1.18.5.3, Table 39). Details regarding SAEs related to pregnancy for Study EBS.AVA.212 are provided in Section 9.1.1 Human Reproduction and Pregnancy Data.

Congenital Abnormalities

Congenital abnormalities were reported in two pregnant subjects in EBS.AVA.212, Subject US(b) (6) and Subject US(b) (6) . Five congenital anomalies were reported in three infants born to these two subjects, with one reported fetal death due to three congenital anomalies. Details regarding the reported congenital abnormalities for EBS.AVA.212 are summarized in Section 9.1.1 Human Reproduction and Pregnancy Data.

Reviewer comment: *A number of SAEs were reported in Study EBS.AVA.212, but none were related to IP. While two SAEs (summarized above) were considered ‘possibly related’ to vaccination with IP per the investigator, follow-up information about pre-existing and concomitant medical conditions that were biologically plausible explanations for the respective SAEs (acute cholecystitis in a subject with gallbladder disease and gallstones and sialadenitis after streptococcal infection) made it highly unlikely that these two SAEs were related to vaccination, as indicated by the Applicant’s assessment of causality (of ‘not related’). No trend or pattern in SAEs reported was seen after AV7909 (or BioThrax) administration. For pregnancy-related SAEs in female subjects, the majority of reported maternal SAEs were related to miscarriage (spontaneous abortion).*

6.1.12.5 Adverse Events of Special Interest (AESIs)

An AESI was defined as any AE having an autoimmune etiology, as provided in Appendix B in the Study protocol for EBS.AVA.212 (STN 125761/0, EBS.AVA.212. Study Protocol, Appendix 16.1.1).

Subjects were monitored for AESIs up to Month 13 (12 months after administration of the last IP dose). From Day 65 through Month 13, confirmed AESIs (as assessed by the DSMB) were recorded on the AE eCRF. The status of ongoing SAEs/AESIs after Day 64 were reviewed at each quarterly safety phone contact to determine any new information and to update the resolution status in the AE eCRF. AESIs were followed by the PI or designee until one or the other condition was met:

- The SAE/AESI was resolved or stable if expected to remain chronic.
- The subject was referred to a specialist or other physician for treatment and follow-up.

The PI or designee followed the subject’s condition, even if the subject was seen by another physician, to obtain information about the diagnosis and outcome and any treatments and medications administered for the event. A DSMB expert who was a board-certified rheumatologist/immunologist adjudicated all potential AESIs to confirm the diagnosis and, if confirmed, to determine the relationship of IP administration to the AESI event and plausibility of IP administration for causation of the event.

Confirmed AESIs reported in EBS.AVA.212 are summarized in Table 15 below.

Table 15. Study EBS.AVA.212: Confirmed Adverse Events of Special Interest (AESIs) by MedDRA System Organ Class (SOC) and Preferred Term (PT) (Safety Population¹)

MedDRA System Organ Class Preferred Term	AV7909 Three Lots Pooled (N=3151) n/%	BioThrax (N=533) n/%
All AESIs	15 (0.5)	2 (0.4)
Related	3 (<0.1)	1 (0.2)
Unrelated	11 (0.3)	1 (0.2)
Unknown	1 (<0.1)	0
Endocrine disorders	3 (<0.1)	0
Basedow's disease	2 (<0.1)	0
Autoimmune thyroiditis	1 (<0.1)	0
Gastrointestinal disorders	2 (<0.1)	0
Celiac disease	1 (<0.1)	0
Ulcerative colitis	1 (<0.1)	0
Musculoskeletal and connective tissue disorders	3 (<0.1)	1 (0.2)
Polymyalgia rheumatica	1 (<0.1)	0
Psoriatic arthropathy	1 (<0.1)	0
Systemic lupus erythematosus	1 (<0.1)	0
Rheumatoid arthritis	0	1 (0.2)
Skin and subcutaneous tissue disorders	7 (0.2)	1 (0.2)
Guttate psoriasis	2 (<0.1)	0
Subacute cutaneous lupus erythematosus	2 (<0.1)	0
Alopecia areata	1 (<0.1)	0
Chronic spontaneous urticaria	1 (<0.1)	0
Diffuse alopecia	1 (<0.1)	0
Lichen planus	0	1 (0.2)

¹Safety Population excludes site US1027.

AESI = Adverse event of special interest; N = Number of subjects in the Safety Population; n = Number of subjects with confirmed AESIs within each group exposed to the treatment; % = n/N*100

MedDRA = Medical Dictionary for Regulatory Activities SOC = System organ class. PT = Preferred term.

SOCs were sorted in alphabetical order and PTs within each SOC were sorted in descending order of percentage.

Adverse events were coded according to MedDRA version 22.0.

Ref: STN 125761/0, EBS.AVA.212, CSR Addendum 2, Table 23, pages 47-48 of 73. Source: Table 14.3.1.10b.

The incidence of confirmed AESIs was low and similar in the combined AV7909 group (15/3151 subjects, 0.5%) and the BioThrax group (2/533 subjects, 0.4%). The majority of AESIs, when adjudicated by the DSMB expert, were deemed unrelated to IP administration but generally due to pre-existing medical conditions or pre-existing abnormal laboratory tests, as summarized in Table 16 below.

Table 16. Study EBS.AVA.212: Summary of Adverse Events of Special Interest (AESIs) and Attribution to Investigational Product (Safety Population)

Patient ID	Age/Race/ Sex/Treatment	AESI Diagnosis	Presence of abnormal baseline labs	Other confounding medical conditions	Number of days of onset post- last vaccination	Attribution of AESI to Investigational Product (IP)
US(b) (6)	43 yrs./White female/ AV7909 Lot 2	Ulcerative colitis	No	NA	179 days	PI: possibly related DSMB: possibly related Applicant: possibly related
US(b) (6)	21 yrs./White male/ AV7909 Lot 2	Alopecia areata	No	NA	255 days	PI: unrelated DSMB: unrelated Applicant: unrelated

Patient ID	Age/Race/ Sex/Treatment	AESI Diagnosis	Presence of abnormal baseline labs	Other confounding medical conditions	Number of days of onset of event post- last vaccination	Attribution of AESI to Investigational Product (IP)
US(b) (6)	37 yrs./White male/AV7909 Lot 1	Autoimmune thyroiditis and hypothyroidism	Yes (elevated anti-thyroid ant body)	NA	100 days	PI: unrelated DSMB: unrelated (due to pre-existing anti-TPO antibodies at baseline) Applicant: unrelated
US(b) (6)	23 yrs./White female/AV7909 Lot 1	Subacute cutaneous lupus erythematosus	Yes (Positive ANA)	NA	29 days	PI: unrelated DSMB: unrelated (due to pre-existing abnormal labs) Applicant: unrelated
US(b) (6)	40 yrs./White female/BioThrax	Rheumatoid arthritis	Yes (Positive ANA)	NA	25 days	PI: unrelated DSMB: unrelated (due to pre-existing abnormal labs) Applicant: unrelated
US(b) (6)	44 yrs./White male/AV7909 Lot 3	Guttate psoriasis	EB NA IgG and EB VCA IgG positive (IgM (-))	Positive EB NA IgG and EB VCA IgG; Upper respiratory infection (URI); Strep throat; Positive ANA	56 days	PI: Probably related (subject's antinuclear antibody (ANA) seroconversion post- third vaccine dose could increase likelihood of developing connective tissue disease (CTD) DSMB: unrelated Applicant: unrelated
US(b) (6)	27 yrs./White female/ AV7909 Lot 2	Guttate psoriasis	No	2 episodes of streptococcal pharyngitis	124 days (13 days after 2 nd strep throat episode)	PI: possibly related (but AESI could also be possibly-related to recent strep infection) DSMB: unrelated Applicant: unrelated
US(b) (6)	19 yrs./White female/ AV7909 Lot 1	Subacute cutaneous lupus erythematosus (SCLE)	No	GERD (omeprazole use for 5 yrs)	35 days (+ ANA); Dx confirmed by skin bx: 10.5 months	PI: possibly related DSMB: unrelated (subject was on omeprazole, a known cause of SCLE) Applicant: unrelated
US(b) (6)	50 yrs./White female/ AV7909 Lot 3	Psoriatic arthropathy	No	Joint pain, History of osteoarthritis	140 days	PI: unrelated DSMB: unrelated Applicant: unrelated
US(b) (6)	49 yrs./White female/ AV7909 Lot 3	Grave's (Basedow's) disease	Yes; (Positive ANA, abnormally low TSH)	NA	76 days	PI: unrelated DSMB: unrelated (causality assessment based upon the subject's baseline TSH and ANA which suggested a pre- existing condition prior to IP exposure) Applicant: unrelated
US(b) (6)	21 yrs./African American female/ AV7909 Lot 3	Grave's (Basedow's) disease	Yes; (abnormally low TSH)	NA	32 days	PI: unrelated DSMB: unrelated (pre-existing) Applicant: unrelated

Patient ID	Age/Race/ Sex/Treatment	AESI Diagnosis	Presence of abnormal baseline labs	Other confounding medical conditions	Number of days of onset of event post- last vaccination	Attribution of AESI to Investigational Product (IP)
US(b) (6)	63 yrs./White male/ AV7909 Lot 1	Polymyalgia rheumatica	No	S/P fall (unspecified date) onto left knee and left shoulder; subsequent pain/stiffness in the hips and shoulders	245 days	PI: unrelated DSMB: unrelated Applicant: unrelated
US(b) (6)	35 yrs./White female/ AV7909 Lot 3	Celiac disease	No	Pre-existing intermittent diarrhea; stool positive for salmonella while subject symptomatic	252	PI: possibly related DSMB: unrelated Applicant: unrelated
US(b) (6)	32 yrs./White female/ AV7909 Lot 1	Chronic spontaneous urticaria	No	NA	47 days	PI: possibly related DSMB: possibly related Applicant: possibly related
US(b) (6)	57 yrs./African American female/ AV7909 Lot 2	Diffuse alopecia	No	NA	2 days	PI: possibly related DSMB: possibly related Applicant: possibly related
US(b) (6)	29 yrs./White male/BioThrax	Lichen planus	No	Seasonal and environmental allergies	172 days	PI: possibly related DSMB: possibly related (lichen planus biopsy proven; occurred quite late after vaccination but adjudicator felt possibly related to IP) Applicant: possibly related
US(b) (6)	40 yrs./White female/BioThrax	Rheumatoid arthritis (RA)	Positive ANA	NA	54 days	PI: possibly related DSMB: unrelated (RF and anti-CCP were normal, tests and PE findings did not meet diagnostic criteria for consistent with RA) Applicant: unrelated

AESI: Adverse event of special interest; yrs.: Years; NA: Not applicable; PI: Principal Investigator; DSMB: Data Safety Monitoring Board
TPO: Thyroid peroxidase; ANA: Antinuclear antibody; EB: Epstein Barr; GERD: Gastroesophageal reflux disease; Dx: Diagnosis; Bx:
Biopsy; TSH: Thyroid stimulating hormone; S/P: Status post; RF: Rheumatoid factor; anti-CCP: Anti-cyclic citrullinated peptide antibody
Ref: Table compiled from STN 125761/0, EBS.AVA.212, CSR, Section 14.3.3. Narratives of Deaths, Other Serious and Certain
Other Significant Adverse Events, pages 1-362. Source: Listings 16.2.7.5.a, and 16.2.7.5.b; STN 125761/0, CSR Addendum 1,
Section 14.3.3.4. Adverse Events of Special Interest, pages 138-188, Source: Listing 16.2.7.1

Of all confirmed AESIs associated with AV7909 administration, three AESIs were adjudicated to be 'possibly related' to receipt of AV7909 (ulcerative colitis in Subject US(b) (6), diffuse alopecia in Subject US(b) (6), and chronic spontaneous urticaria in Subject US(b) (6). In subjects who received BioThrax, one confirmed AESI of lichen planus was adjudicated as 'possibly related' to treatment (Subject US(b) (6). Exclusion of site US1027 and reanalysis using the revised safety datasets did not alter any of the AESI findings in EBS.AVA.212 (STN 125761/0/27).

Reviewer comment: *The incidence of AESIs after vaccination with AV7909 and BioThrax was low and comparable in frequency (0.5% vs. 0.4%) and even lower when accounting for relatedness of the AESI to vaccination (3/3151 or <0.1% for AV7909 vs. 1/533 or 0.2% for BioThrax). There was no distinct clinical pattern for the AESIs reported in EBS.AVA.212, as confirmed by the DSMB expert, and the onset intervals spanned a wide time range. The majority of AESIs reported in AV7909 vaccinated subjects were adjudicated by the DSMB expert as 'unrelated' to treatment even when the PI believed that the AESI may have been 'possibly related to treatment' (12/15 or 80% confirmed AESIs), based on the presence of pre-existing medical conditions that were mechanistically plausible etiologies for the reported AESIs. After review of each individual AESI narrative and MedWatch report, the clinical reviewer agrees with the DSMB adjudicator's basis for determining AESI causality, especially in the setting of documented pre-existing laboratory tests and medical signs or symptoms that were consistent with underlying autoimmune disease prior to receipt of vaccine.*

6.1.12.6 Clinical Test Results

Minor fluctuations from mean baseline values in both the combined AV7909 group and the BioThrax group were seen for clinical chemistry, hematology, and urinalysis. No clinically meaningful changes by visit were observed, and there were no important treatment group differences noted in the mean values by study visit, nor any significant changes in range or shift, as seen in laboratory tables. Similarly, for autoantibody testing (RF and TSH), there were no clinically meaningful changes from Day 1 observed or significant treatment group differences in the proportion of subjects with positive or negative autoantibodies, or mean values by study visit for RF or TSH.

Post-baseline shifts that occurred in $\geq 10\%$ in either the combined AV7909 group or BioThrax group included autoantibodies: from negative at baseline to positive at Day 64 in 317/3299 (13.1%) subjects in the combined AV7909 group and in 56/558 (13.6%) subjects in the BioThrax group. There were no clinically meaningful changes from Day 1 observed or significant treatment group differences in the proportion of subjects with positive or negative autoantibodies, or mean values by study visit for RF or TSH.

Reviewer comment: *The clinical significance of post-baseline shifts for autoantibodies is unknown in otherwise healthy individuals but likely represent spurious findings; most of these subjects were asymptomatic for autoimmune disease.*

AEs associated with hematology, clinical chemistry, or urinalysis parameters were infrequent, and none occurred in $\geq 1\%$ of subjects in either the combined AV7909 group or the BioThrax group (STN 125761/0, EBS.AVA.212, CSR, Table 14.3.1.4.1).

The incidence of abnormal PE findings at Day 64 occurred in similar frequencies between the combined AV7909 group (257/3299 subjects, 7.8%) and the BioThrax group (52/558 subjects, 9.3%) (STN 125761/0, EBS.AVA.212, Table 14.3.4.8.1). No clinically significant PE findings, and no important treatment group differences were observed between the combined AV7909 group and the BioThrax group. There were no AEs associated with PE findings. Relatively minor fluctuations from baseline VS were recorded for both the combined AV7909 group and the BioThrax group. No clinically meaningful changes from baseline were observed, and there were no meaningful differences noted between the combined AV7909 group and the BioThrax group.

Reviewer comment: *There were no clinically significant laboratory test or physical exam findings in AV7909 or BioThrax vaccinated subjects in EBS.AVA.212.*

6.1.12.7 Dropouts and/or Discontinuations

AEs (all AEs, IP-related and unrelated) leading to discontinuation of vaccination or study withdrawal, were infrequent and occurred in similar proportions in the combined AV7909 group (72/3299 [2.3%] and 1/3151 [$<0.1\%$] subjects, respectively) and the BioThrax group (14/533 [2.6%] and 0 subjects, respectively). IP-related AEs leading to treatment discontinuation were likewise infrequent and similar in frequency between the combined AV7909 group (43/3151 [1.4%]) and the BioThrax group (9/533 [1.7%]) (STN 125761/0/27, EBS.AVA.212, CSR Addendum 3, Table 11, pages 28-31 of 57, Source: Table 14.3.1.11b., Listing 16.2.7.3). There was one event (PT of menorrhagia) that led to study withdrawal; this event was considered 'not related' to AV7909 vaccination (data not shown; STN 125761/0/27, EBS.AVA.212, CSR Addendum 2, Table 22, pages 44-47 of 73). None of the TEAEs leading to IP discontinuation occurred in more than 1.1% within the combined AV7909 group or the BioThrax group.

Reviewer comment: *Subject dropouts and discontinuation of treatment was low across all treatment groups (AV7909 and BioThrax), including those that were due to IP administration.*

6.1.13 Study Summary and Conclusions

Immunogenicity Summary and Conclusion

Primary immunogenicity analysis in Study EBS.AVA.212 comprised an evaluation of (1) AV7909 lot consistency and (2) AV7909 immunogenicity (by TNA NF_{50}) at Day 64; with two co-primary endpoints assigned to each of these two immunogenicity assessments (four co-primary endpoints). The pre-specified criteria for two AV7909 immunogenicity co-primary endpoints at Day 64 were met, thereby demonstrating both lot consistency across the three AV7909 lots and a protective level of immunogenicity at 7 weeks (Day 64) after completion of the two-dose IM schedule of AV7909 (Days 1 and 15) in healthy adults (18 to 65 years of age).

While all four co-primary immunogenicity endpoints met success criteria, BioThrax immune responses were observed to be lower than expected, in the noninferiority comparison with AV7909, compared to BioThrax TNA NF_{50} responses observed in prior studies of BioThrax (Study EBS.AVA.006 in BLA STN 103821/5344). The Applicant was unable to determine the cause of this discrepancy; CMC and potency tests to assess CMC performance of the BioThrax lots used in Study EBS.AVA.212 did not identify a product-related reason for this finding. Reanalysis of the four, co-primary immunogenicity endpoints, with revised datasets that excluded site US1027 (done because of data integrity issues at site US1027), AdAM and SDTM dataset issues, and additional inaccuracies identified in the ADFACE and ADAE datasets during BLA review resulted in no significant numerical changes in the immunogenicity parameters tested and did not affect immunogenicity conclusions in the study. Subgroup analysis of immunogenicity results indicated somewhat higher immune responses in younger subjects (18-30 years of age). Subgroup analysis of immunogenicity did not change appreciably after data from site US1027 were excluded.

In summary, AV7909 met all immunogenicity success criteria, with effectiveness and lot consistency demonstrated in Study EBS.AVA.212.

Safety Summary and Conclusion

Injection site reactions were relatively frequent in both the AV7909 and BioThrax groups (e.g., tenderness, pain, and myalgia). Local and systemic reactogenicity, when assessed by e-diary, indicated slightly greater local reactogenicity in BioThrax vaccinated subjects and slightly greater systemic reactogenicity in AV7909 vaccinated subjects. Nonetheless, the severity of both local and systemic reactions was generally Grade 1 or 2; Grade 3 reactions were very infrequent. There were no Grade 4 local or systemic reactions reported in EBS.AVA.212. When assessed in-clinic, local and systemic reactions were markedly lower in frequency for both the AV7909 and BioThrax groups. Injection site reactions appeared to be more frequent and severe in the younger age group (18-30 years of age) and slightly higher in female subjects.

The most common TEAEs were related to injection site reactions, with tenderness, pain, and muscle ache (myalgia) the most common solicited AEs post-vaccination. There were no AE patterns or safety signals detected in Study EBS.AVA.212 for AV7909 (or BioThrax) vaccinated subjects. All reported SAEs were unrelated to vaccination. AESIs were infrequent and were balanced between the AV7909 and BioThrax arms, with no discrete trend or pattern in AESIs observed. Reanalysis of safety data with revised safety datasets and exclusion of site US1027 did not alter safety findings or conclusions regarding safety endpoints assessed in EBS.AVA.212.

In summary, AV7909 appeared to be generally well-tolerated in study subjects with no significant safety concerns identified for AV7909 in Study EBS.AVA.212.

6.2 Trial #2

EBS.AVA.210 (NCT04067011): A Phase 2 Drug-Vaccine Interaction Study to Examine Whether Co-administration of AV7909 with Ciprofloxacin or Doxycycline Affects Antibiotic Pharmacokinetics (PK) or AV7909 Immunogenicity in Healthy Adults

6.2.1 Objectives

Primary Objective:

- To evaluate the PK profiles of ciprofloxacin or doxycycline when administered orally (PO) prior to and following the IM administration of a 2-dose schedule of AV7909, administered two weeks apart.

Secondary Objectives:

- To assess the safety of concurrent administration of oral ciprofloxacin or doxycycline and two doses of AV7909 administered IM.
- To evaluate the Day 37 immune response using the TNA assay following two IM doses of AV7909 with and without the concurrent oral administration of ciprofloxacin or doxycycline.

Reviewer comment: *The purpose of this study was to identify any potential effects of AV7909 vaccination on the PK of ciprofloxacin or doxycycline—two first-line antimicrobials for PEP after anthrax exposure¹⁰ and to determine any potential effects of ciprofloxacin or doxycycline therapy on the immunogenicity of AV7909, since antimicrobials and anthrax vaccine would be given concomitantly for PEP against inhalational anthrax after an anthrax attack or potential anthrax exposure event.*

6.2.2 Design Overview

This was a randomized, open-label, Phase 2, multicenter study to investigate potential interactions (i.e., interference effects) of AV7909 with ciprofloxacin or doxycycline when administered concomitantly. The potential effect of AV7909 vaccination on ciprofloxacin or doxycycline serum levels was investigated by evaluating the changes in the single-dose and steady-state PK profiles of ciprofloxacin or doxycycline before and after vaccination with a two-dose series of AV7909. The effect of ciprofloxacin or doxycycline dosing on the immunogenicity of AV7909 was investigated by evaluating whether TNA assay levels two weeks following the final dose of the two-dose AV7909 vaccination series is affected by concomitant dosing with oral ciprofloxacin or doxycycline. Both ciprofloxacin and doxycycline were chosen for use in this study because they are first-line therapies recommended by the ACIP for anthrax PEP.¹⁰

Reviewer comment: *In keeping with FDA recommendations regarding the clinical development of AV7909 for PEP against inhalational anthrax (IND 14451, EOP 2 Meeting Minutes, Comment 9; 31 August 2015), Study EBS.AVA.210's design was similar to Study EBS.AVA.009 (NCT01753115)—the antimicrobial (ciprofloxacin)-vaccine interaction clinical study used to support licensure of BioThrax for the PEP indication. Although it was confirmed with FDA that ciprofloxacin was the only antimicrobial that required evaluation of interaction with AV7909 (EOP 2 Meeting Minutes, Comment 10; 31 August 2015), Study EBS.AVA.210 also evaluated the interaction of AV7909 with an additional antibacterial, doxycycline, as both antibacterial drugs are stockpiled for PEP of anthrax disease and may be used concomitantly with AV7909 in a mass exposure event.*

Eligible healthy males and females 18-45 years of age were randomized 1:1:1 into one of the following three IP groups; as shown in Table 17, below.

Table 17. EBS.AVA.210: Study Groups

IP Group	Treatment Group	Treatment	Planned Sample Size (N)
1. Ciprofloxacin	1A	AV7909 + ciprofloxacin (with PK assessment)	40
1. Ciprofloxacin	1B	AV7909 + ciprofloxacin (without PK assessment)	30
2. Doxycycline	2A	AV7909 + doxycycline (with PK assessment)	40
2. Doxycycline	2B	AV7909 + doxycycline (without PK assessment)	30
3. AV7909	3	AV7909 only	70

IP = investigational product; N = number of subjects; PK = pharmacokinetic.
Ref: STN 125761/0, CSR EBS.AVA.210, Table 2, page 19 of 129.

The first 40 of the 70 subjects randomized to receive AV7909 in combination with either ciprofloxacin or doxycycline were assigned to treatment Groups 1A or 2A, respectively. Subjects randomized to receive AV7909 alone were assigned to Group 3. Once there were approximately 40 subjects randomized into each of the three IP groups (Group 1A, Group 2A, and Group 3), subsequent randomized subjects were then assigned to Group 1B, Group 2B, or Group 3. Randomization was stratified by site.

Reviewer comment: *The randomization procedures were acceptable. The study was unblinded (open-label design).*

6.2.3 Population

Key Eligibility Criteria

Key Inclusion Criteria:

Same as for Study EBS.AVA.212 (see Section 6.1.3)

Key Exclusion Criteria:

Same as for Study EBS.AVA.212, with the following additional criteria:

- A screening clinical laboratory test result greater than the central laboratory's upper limit of normal (ULN) for aspartate aminotransferase (AST), alanine aminotransferase (ALT), random glucose, total bilirubin, blood urea nitrogen (BUN), or creatinine. Other serum chemistry parameters that were not within the reference range were not considered exclusionary unless deemed clinically significant by the PI.
- History of allergic reaction or intolerance to quinolone antimicrobials or any medical condition that contraindicated the use of ciprofloxacin, including and not limited to vascular disorders, tendon disorders, certain genetic connective tissue disorders (e.g., Marfan and Ehlers-Danlos syndrome), prolongation of QT interval, seizures, peripheral neuropathy, increased risk of *C. difficile* infection.
- History of allergic reaction or intolerance to tetracycline antibiotics or any medical condition that contraindicated the use of doxycycline, including an increased risk of *C. difficile* infection, increases in BUN, or an increased sensitivity to direct sunlight or ultraviolet radiation resulting in erythema.
- Need for any of the prohibited medications (see Prohibited Medications, below).
- Positive urine drug screen result, any evidence of ongoing drug abuse or dependence (including alcohol), or recent history (over the past five years) of treatment for alcohol or drug abuse.

Prohibited Medications

An extensive list of prohibited medications (including vaccines, biologics, and investigational agents) for all enrolled study subjects was provided (STN 125761/0, EBS.AVA.210, CSR, Table 4, page 32 of 129).

The general categories of therapeutic agents that was excluded in all subjects comprised the following:

- Anti-inflammatory or antipyretic medications: prohibited within 24 hours prior to or after vaccination.
- Aspirin withheld on each day of vaccination; with allowance for resumed use the following day.
- Vaccines: prohibited until 30 days after final vaccination (live) or two weeks after final vaccination (inactivated).
- Immunosuppressive therapy.
- Cytotoxic therapy.
- Investigational medicinal products.
- Blood thinners and anti-coagulants.
- Parenteral immunoglobulins or blood products.

In addition, a separate prohibited medication list was provided in the study protocol for subjects who received ciprofloxacin (STN 125761/0, EBS.AVA.210, Study Protocol, Section 6.5.1.2, page 57 of 420) and doxycycline (STN 125761/0, EBS.AVA.210, Study Protocol, Section 6.5.1.3, page 57 of 420), respectively.

Reviewer comment: *The list of prohibited medications for all subjects, along with those provided for subjects additionally receiving either ciprofloxacin or doxycycline, is appropriate and reflects known and suspected interactions between the listed medications/biologic agents/vaccines and AV7909 and/or the respective antimicrobial.*

Dietary restrictions, activity restrictions, and use and/or timing of over-the-counter (OTC) products were pre-specified to minimize impact on absorption of antibiotics.

Reviewer comment: *The list of foods and OTC products that were to be avoided prior to dosing with antibiotics, so as not to potentially alter the absorption and PK of the respective antibiotic, was reasonable and consistent with procedures employed in PK Study EBS.AVA.009, for the PEP indication of BioThrax (STN 103821/5344). The proposed activity restriction during PK sampling was reasonable.*

6.2.4 Study Treatments or Agents Mandated by the Protocol

Study treatments administered in EBS.AVA.210 comprised the following:

- **AV7909 (b) (4) AVA + (b) (4) CpG 7909**; Manufacturing Lot# 100001A, (b) (4) Lot# (b) (4) : administered IM in the deltoid muscle of alternating arms to all subjects (Groups 1 to 5) on Day 8 and Day 23.
- **Ciprofloxacin** (500 mg per os (po; orally) q 12 hr.; Manufacturing Lot#: C900163, Packaging Lot# B190330): administered to subjects in Group 1 on Days 4-9, Days 22-24, and Days 31–37.
- **Doxycycline** (100 mg po q 12 hr.; Manufacturing Lot# 770424A, Packaging Lot# B190331): administered to subjects in Group 2 on Days 2-9, Days 22-24, and Days 32–38.

Reviewer comment: *The evaluated dosing regimens of ciprofloxacin and doxycycline would be used for PEP in the event of anthrax exposure.*

6.2.5 Directions for Use

AV7909 was administered to all subjects in all groups as a 0.5 mL IM vaccination in alternate arms as a two-dose Day 1 and 15 dosing schedule (given on Days 8 and 23). Vaccinations were administered in the clinic by authorized personnel once AEs had been assessed, vitals taken, and a symptom-directed PE conducted. Female subjects who had not demonstrated a follicle-stimulating hormone (FSH) level >30 mIU/mL required confirmation of a negative urine pregnancy test prior to receipt of AV7909.

Subjects in Groups 1A and 2A were to receive their first AV7909 vaccination after the 12-hour antibiotic PK sample (and after evening dose of the antibiotic) on Day 8, and their second AV7909 vaccination on Day 23. Ciprofloxacin (500 mg po q 12 hours) was to be administered to subjects in Group 1 on Days 4 through 9, Days 22 through 24, and Days 31 through 37.

Antibiotics were to be administered in three courses by site staff at in-clinic visits or by subjects when at home. Approximately the first 40 subjects in Group 1A (with PK assessments) were to self-administer 14 of the 31 ciprofloxacin doses; the remainder of doses were to be administered by site staff during in-clinic visits. The remaining subjects (approximately 30) in Group 1B (without PK assessment), were to self-administer 29 of the 31 ciprofloxacin doses, while the remaining two doses were to be administered by site staff during in-clinic visits.

Doxycycline (100 mg po q 12 hours) was administered only to subjects in Group 2. A total of 35 ±2 doses was administered in three courses. Group 2A subjects nominally received 20 of their 35 doxycycline doses in the clinic whereas Group 2B nominally received two of their 35 doxycycline doses in the clinic, and the remaining doses were taken at home.

Antibiotic doses were to be taken at the same time (± 30 min) each day. Doses administered in the clinic were recorded by the staff with the date and time of administration. Doses self-administered at home were to be recorded by the subject with time of administration in their diary.

Reviewer comment: *For both antibiotics, administration occurred on an intermittent daily rather than a continuous daily dosing schedule (per the licensed anthrax PEP ciprofloxacin and doxycycline dosing schedules in their respective package inserts), with the assumption that intermittent dosing would achieve the required single dose and steady state concentrations needed prior to and following the vaccination schedule. The intermittent dosing schedule was also chosen to minimize any adverse effects from ciprofloxacin and doxycycline. For ciprofloxacin, dosing was similar to that in Study EBS.AVA.009 (doxycycline not evaluated), to support licensure of BioThrax for PEP against disease due to anthrax exposure (STN 103821/5344). Dosing procedures were appropriate for the intent of this antimicrobial-vaccine interaction study.*

6.2.6 Sites and Centers

EBS.AVA.210 was conducted at four US Sites.

6.2.7 Surveillance/Monitoring

Effectiveness assessments in EBS.AVA.210 comprised antimicrobial (ciprofloxacin and doxycycline) PK evaluations and an evaluation of the post-vaccination immune response of the AV7909 PEP dosing schedule (Week 0 and 2 IM administration).

Pharmacokinetic Evaluation

Ciprofloxacin PK in Group 1A was measured on Days 4, 8, 31, and 35. Ciprofloxacin pre-dose or trough values were measured prior to the morning doses of ciprofloxacin on Days 4, 5, 6, 7, and 8; and on Days 31, 32, 33, 34, and 35. On days where a PK assessment was performed, blood samples for measurement of ciprofloxacin concentrations were collected at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10 and 12 hours post-dose (± 5 minutes through hour 4, \pm then 15 minutes).

Doxycycline PK in Group 2A was measured on Days 2, 8, 32, and 38. Doxycycline pre-dose or trough values was measured prior to the morning doses of doxycycline on Days 2, 3, 4, 5, 6, 7, and 8; and on Days 32, 33, 34, 35, 36, 37, and 38. On days where a PK assessment was performed, blood samples for measurement of doxycycline concentrations were collected at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10 and 12 hours post-dose (± 5 minutes through hour 4, \pm then 15 minutes).

Reviewer comment: *The PK sampling procedures (e.g., parameters and time points assessed) for EBS.AVA.210 were previously reviewed under IND 14451.A88 (study protocol for EBS.AVA.210; unchanged from the protocol submitted under A88); and determined to be acceptable.*

Immunogenicity Evaluation

Blood samples for the determination of TNA titers were collected on all subjects on Day 1 (baseline) and Day 37, which was two weeks after the last vaccination (± 1 day each).

All immunogenicity laboratory samples were evaluated using a validated TNA assay. Specific procedures related to collection, processing, storage, and shipment of the samples were provided to the sites. The TNA assay used in this trial had been validated by (b) (4) under National Institute of Allergy and Infectious Diseases (NIAID) sponsorship. The TNA assay results were reported as the reciprocal of a serum sample dilution that resulted in 50% neutralization of LT cytotoxicity (50% effective dilution; ED₅₀). To standardize assay results, the results were divided by the ED₅₀ of a serum reference standard (AVR801), and the resulting ratio was reported as a 50% neutralization factor or NF₅₀.

Reviewer comment: *The TNA assay used to assess NF₅₀ for EBS.AVA.210 was the same assay used for measuring TNA responses to BioThrax under STN 103821/5344 to support licensure of BioThrax for PEP, and likewise the same TNA assay as used to assess the immune response to AV7909 in Study EBS.AVA.212.*

Safety monitoring comprised the following:

- In-clinic evaluation of medical history (Screening visit only), PE with VS assessment (complete PE at Screening, targeted PE at Study Days 1, 8, 51 and EWW) and review of concomitant medication use.
- In-clinic assessment of AEs including TEAEs, SAEs and AESIs of potential autoimmune etiology (latter listed in Appendix B).
- Subject evaluation of solicited local and systemic reactogenicity via e-diary from the first day of receipt of IP through Day 45 (one week following last receipt of IP by any group). Reactogenicity symptoms and severity scales utilized in Study EBS.AVA.210 were identical to those used in EBS.AVA.212. E-diary data collected for 7 days post-vaccination were reviewed at each clinic visit and at follow-up telephone contacts by the PI or designee.
 - Subjects with injection site or systemic reactions beyond seven days were prompted to continue daily e-diary entries until resolved for at least two consecutive days.
- Long-term safety follow-up telephone calls at 3 months (Day 114 ±14 days), 6 months (Day 205 ±14 days), 9 months (Day 296 ±14 days), and 12 months (Day 383 ±14 days) were conducted after the last vaccination, to collect data on SAEs or on AESIs of potential autoimmune etiology.
- Laboratory testing (serum chemistry, hematology, urinalysis) performed at Screening and Day 51 (Final clinic visit) or EWW. Blood samples for autoantibody assessment were taken at Day 1 and 37, for testing of antinuclear antibodies (ANA), RF, and anti-dsDNA antibodies.
- Serum pregnancy testing performed at the Screening visit for WOCBP to determine subject eligibility, with confirmation of a negative urine pregnancy test for WOCBP prior to vaccination.

AE severity, laboratory tests for select analytes, and VS results were assessed using FDA's Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials.³³

Subjects who withdrew or discontinued the study before the final visit on Day 51 were asked to complete an EWW and asked to participate in long-term safety follow-up via phone calls.

Individual subject and study stopping rules (STN 125761/0, EBS.AVA.210, CSR, Section 9.3.3.2.1, pages 25-26 of 129) were provided by the Applicant (discussed under 'Treatment Modifications'). A DSMB provided independent safety oversight and was notified of significant

AEs, as determined by the Applicant's MM (e.g., SAEs, severe AEs recorded on the eCRF, potential AESIs of autoimmune etiology). Per the DSMB Charter (STN 125761/0, EBS.AVA.210, CSR, Appendix 16.1.13. page 4-28 of 281) the DSMB made recommendations regarding the safety of continued subject enrollment and dosing. The DSMB comprised three voting members, with at least one member having expertise in the diagnosis and treatment of autoimmune diseases to serve as an adjudicator for AESIs. The operations of the DSMB were detailed in a DSMB charter which was finalized prior to screening the first subject.

6.2.8 Endpoints and Criteria for Study Success

Response Criteria/Case Definition/Endpoint:

Co-Primary Endpoints:

- Area under the curve from 0 to 12 hours (AUC_{0-12h}) and maximum concentration (C_{max}) for ciprofloxacin on Days 8 and 35.
- Area under the curve from 0 to 12 hours (AUC_{0-12h}) and maximum concentration (C_{max}) for doxycycline on Days 8 and 38.

Secondary Endpoints:

- To assess the safety of concurrent administration of oral ciprofloxacin or doxycycline and two doses of AV7909 administered IM.
- To evaluate the Day 37 immune response using the TNA assay following two IM doses of AV7909 with and without the concurrent oral administration of ciprofloxacin or doxycycline.

Reviewer comment: *The proposed study endpoints were acceptable.*

6.2.9 Statistical Considerations & Statistical Analysis Plan

The SAP utilized noncompartmental analytic methods to characterize the following PK parameters for ciprofloxacin and doxycycline:

- Maximum observed concentration: C_{max}
- Time to C_{max} and time of maximum observed concentration (T_{max})
- half-life: $T_{1/2}$
- Apparent elimination rate: K_{el}
- Area under the serum concentration-time curve (12 hours): AUC_{0-12h}
- Area under the first moment curve: AUMC
- Mean residence time: MRT
- Area under the serum concentration-time curve extrapolated to infinity: $AUC_{0-\infty}$
- Rate of absorption: R_{abs}

Sample Size Considerations

Antibiotic PK: Assessment of the effect of vaccination with AV7909 on the PK of either antibiotic was made using the geometric mean of the within-subject ratio of C_{max} and AUC_{0-12h} for either ciprofloxacin or doxycycline before (Day 8) vs. after (Day 35 or Day 38) AV7909 vaccination.

The equivalence (no interaction) margin for the ratio of (0.80, 1.25), was compared with the 90% CI for the geometric mean ratio (GMR). If the correlation of variation with the within-subject ratio was 30% and the true ratio was 0.95, 27 subjects would allow for 90% power at a significance level of 0.05 while 34 subjects would provide 95% power. Therefore, a group size of 40 subjects

in each of the antibiotic PK groups (Group 1 and Group 3) was planned to allow for up to 30% of subjects being excluded from the PK population that would still allow for adequate powering for antibiotic PK evaluation.

Immunogenicity: Sample sizes of 53 in each cohort (AV7909 alone vs. AV7909 + antibiotic [either ciprofloxacin or doxycycline]) would provide 90% power at the 0.05 level for the non-inferiority test (defined as the LB of the two-sided 95% CI of the ratio ≥ 0.5), if the true ratio of geometric means was 0.85 and the correlation of variation of NF_{50} values between subjects in the same group was 100%. A cohort size of 70 was planned to allow for up to 20% of subjects to be excluded from the immunogenicity population.

For the primary PK analysis, assuming a 20% CV for both C_{max} and AUC_{0-12h} and a lack of an interaction, i.e., the true GMRs for C_{max} and AUC_{0-12h} ranged between 95% and 105%, a sample size of 20 subjects would have 80% power at an α level of 0.05 to obtain 90% CIs within 80.00% to 125.00%, i.e., declaring equivalence or no interaction. Enrollment of 30 subjects was thought to account for an adequate number of dropouts to successfully allow analysis of all subjects who completed both pre- and post-vaccination assessments.

Reviewer comment: *Based on the assumptions cited above for both the PK analysis of ciprofloxacin/doxycycline and immunogenicity evaluation post-AV7909 administration, the proposed sample size for each study cohort was reasonable. Please refer to the statistical reviewer's assessment of study powering for further information about sample size and the study's design.*

Criteria for Demonstration of Non-inferiority

- For the primary PK endpoints, equivalence (no interaction) was demonstrated if the 90% CIs for the within-subject ratios (before and after vaccination) of AUC_{0-12h} and C_{max} was completely contained within the equivalence boundary of [0.80, 1.25].
- For the secondary immunogenicity endpoint, non-inferiority (no interaction) was demonstrated if the lower limit of the two-sided 95% CIs for the ratio of the geometric mean NF_{50} values at Day 35 between subjects who received the AV7909 + ciprofloxacin regimen or AV7909 + doxycycline, and those who received only AV7909, was greater than the noninferiority margin of 0.5.

Effect of AV7909 on Ciprofloxacin Pharmacokinetics

To examine the effect of AV7909 vaccination on the steady state PK of ciprofloxacin, the trough values for Days 4-8 and Days 31-35 were analyzed to demonstrate that ciprofloxacin concentrations achieved steady state on Day 8 and Day 35. Then the C_{max} and AUC_{0-12h} values for ciprofloxacin determined for Group 1 subjects on Day 35 (following two doses of AV7909) were compared to those determined on Day 8 (prior to AV7909 vaccination). Point estimates and 90% CIs were calculated for the GMRs. If the CIs fell within 0.80 and 1.25, it was concluded that AV7909 vaccination did not significantly influence the steady state C_{max} and AUC_{0-12h} values of ciprofloxacin.

Effect of AV7909 on Doxycycline Pharmacokinetics

To examine the effect of AV7909 vaccination on the steady state PK of doxycycline, the trough values for Days 2-8 and Days 32-38 were analyzed to demonstrate that doxycycline concentrations achieved steady state on Day 8 and Day 38. Then the C_{max} and AUC_{0-12h} values for doxycycline determined for Group 3 subjects on Day 38 (following two doses of AV7909) were compared to those determined on Day 8 (prior to AV7909 vaccination). Point estimates and 90% CIs were calculated for the GMRs. If the CIs were within 0.80 and 1.25, it was

concluded that AV7909 vaccination did not significantly influence the steady state C_{max} and AUC_{0-12h} values of doxycycline.

Effect of Ciprofloxacin or Doxycycline on AV7909 Immunogenicity

To evaluate whether the administration of ciprofloxacin affects the immunogenicity of AV7909, TNA NF_{50} values two weeks after the second dose of AV7909 were compared between the cohort of subjects that received both ciprofloxacin and AV7909, i.e., the combination of Group 1 + Group 2, also known as the ciprofloxacin test cohort, and the cohort who received AV7909 only, i.e., Group 5 or the reference cohort. Point estimates and two-sided 95% lower CIs were constructed for the ratio (test: reference) of geometric means. If the LB of the two-sided 95% lower CI was greater than 0.5 (the non-inferiority margin) it was concluded that the immune response in the cohort that received AV7909 plus ciprofloxacin is non-inferior to the cohort that received AV7909 alone and thus that ciprofloxacin did not demonstrably affect the immunogenicity of AV7909.

The same evaluation was conducted to determine if the administration of doxycycline affects the immunogenicity of AV7909 in that TNA NF_{50} values two weeks after the second dose of AV7909 were compared between the cohort of subjects that received both doxycycline and AV7909, i.e., the combination of Group 3 + Group 4, also known as the doxycycline test cohort, and the cohort who received AV7909 only, i.e., Group 5 or the reference cohort. Point estimates and two-sided 95% lower CIs were constructed for the ratio (test: reference) of geometric means. If the LB of the two-sided 95% lower CI was greater than 0.5 (the non-inferiority margin) it was concluded that the immune response in the cohort that received AV7909 plus doxycycline is non-inferior to the cohort that received AV7909 alone and thus that doxycycline did not demonstrably affect the immunogenicity of AV7909.

Reviewer comment: *The statistical approach for evaluating interference between AV7909 and antimicrobial therapy (i.e., ciprofloxacin) in EBS. AVA.210 was the same as that applied in a similar study for BioThrax (EBS.AVA.009), under STN 103821/5344. The addition of the doxycycline arm addresses ACIP anthrax working group concerns regarding evaluation of interference between doxycycline and AV7909 (since doxycycline is also likely to be used as antimicrobial prophylaxis in a mass anthrax event).*

No changes were made to the planned analyses after the finalization of the SAP. The SAP was finalized prior to clinical database lock (to include data up to and including last subject's last in-clinic visit [Day 51; i.e., four weeks after second vaccination]).

6.2.10 Study Population and Disposition

A total of 210 subjects were randomized to receive either AV7909 plus ciprofloxacin, AV7909 plus doxycycline, or AV7909 alone.

6.2.10.1 Populations Enrolled/Analyzed

Study Populations comprised the following:

- ITT Population: defined as all subjects who were randomized. Subject disposition and baseline demographics were summarized by IP group and overall, for the ITT Population according to the group into which the subject was randomized.
- Safety Population: defined as all randomized subjects who received at least one dose of antibiotic or AV7909. Subjects were included in the IP group according to the treatment they received.

- PK Population: comprised all subjects in Group 1A and Group 2A who:
 - Received two doses of AV7909 according to the protocol (i.e., correct dose, no temperature excursion, and within the study-specified windows),
 - If randomized to Group 1A, received at least five of the seven in-clinic ciprofloxacin doses between Day 4 and the morning of Day 8, and between Day 31 and the morning of Day 35,
 - If randomized to Group 2A, received at least seven of the nine in-clinic doxycycline doses between Day 2 and the morning of Day 8, and between Day 32 and the morning of Day 38,
 - Had adequate data for calculation of the PK parameters at the Day 8 (both Groups 1A and 2A) and Day 35 (Group 1A) or Day 38 (Group 2A) visits, and
 - Had no protocol deviations/events that affected ciprofloxacin or doxycycline steady-state PK assessment or immunogenicity results.

The PK Population was used for all PK analyses. Subjects were included in the treatment group (Group 1A or Group 2A) according to the antibiotic received.

- Immunogenicity Population: defined as all randomized subjects who:
 - Received two doses of AV7909 according to the protocol (e.g., correct dose, no temperature excursion, and within the study-specified windows),
 - Had a valid immunogenicity (TNA) result on Day 1 (pre-vaccination) with no evidence of previous exposure to anthrax or anthrax vaccine (i.e., TNA below the limit of detection of 0.059),
 - Had a valid immunogenicity (TNA) result at Day 37 within the study-specified window, and
 - Took at least 50% of the protocol-specified antibiotic doses (for subjects in Group 1 or Group 2).

The Immunogenicity Population was used for the immunogenicity analyses. Subjects were included in the Immunogenicity Population group (Groups 1, 2, and 3) according to the treatment they received.

6.2.10.1.1 Demographics

Subject demographics for the ITT population in Study EBS.AVA.210 indicate that no meaningful across-treatment differences observed in subject demographics. The overall mean (SD) age of subjects was 32 (± 8) years, and age ranged from 18-45 years. There were somewhat more subjects (n=120, 57.1%) in the 31 to 45-year-old age group, than subjects in the younger age group (i.e., 18 to 30-year-olds; n=90, 42.9%). There were more female subjects (n=134, 63.8%) enrolled in the study than male subjects (n=76, 36.2%). The majority of subjects were White (n=137, 65.2%) and were non-Hispanic or non-Latino (n=179, 85.2%).

Baseline characteristics as measured by height, weight, and BMI were likewise similar across treatment groups, with a BMI range of 25.9-28.6 kg/m².

Reviewer comment: *There were no appreciable subject demographic or baseline characteristic differences seen across the study groups for EBS.AVA.210. The study groups were generally balanced with respect to demographic and baseline factors.*

6.2.10.1.2 Medical/Behavioral Characterization of the Enrolled Population

The most frequently reported medical conditions (in order of highest incidence) across study groups comprised seasonal allergy (range 9.4-14.5%), drug hypersensitivity (range 1.6-17.7%) female sterilization (range 7.8-14.1%), anxiety (range 4.7-11.6%), and myopia (range 6.5-10.9%). Subjects with at least one medical history finding were highest in Group 1 (44; 71.0% of subjects), followed by Group 2 (42; 65.6% of subjects), and then Group 3 (36; 56.3% of subjects).

Reviewer comment: *Subjects in Group 1 generally reported more medical conditions than subjects from Groups 2 and 3. The nature of the reported medical conditions (seasonal allergy, drug hypersensitivity, and anxiety) were unlikely to affect the PK and/or immunogenicity results.*

Concomitant Medication Use

The most frequently used (≥5%) concomitant medication overall was ibuprofen (n=20, 10.5%), followed by progestogens (n=12, 6.3%), paracetamol (n=11, 5.8%), and fixed combinations of progestogens and estrogens (n=11, 5.8%); as provided in Table 4 of CSR Addendum 1 (data not shown; STN 125761/0, EBS.AVA.210, CSR Addendum 1, page 14 of 35; Source: Table 14.1.7.4a). A total of 92 subjects (48.4%) received a concomitant medication; there was no meaningful difference in the proportion of subjects with concomitant medication use across treatment groups (Group 1 [n=32, 51.6%], Group 2 [n=27, 42.2%], and Group 3 [n=33, 51.6%]).

Reviewer comment: *The frequency of concomitant medication use was generally similar across treatment groups.*

6.2.10.1.3 Subject Disposition

Table 18 below provides a summary of subject disposition for randomized subjects across all treatment groups.

Table 18. EBS.AVA.210: Subject Disposition (Randomized Subjects)

Disposition	Cipro + AV7909 Group 1A n (%)	Cipro + AV7909 Group 1B n (%)	Cipro + AV7909 Group 1 (1A + 1B) n (%)	Doxy + AV7909 Group 2A n (%)	Doxy + AV7909 Group 2B n (%)	Doxy + AV7909 Group 2 (2A + 2B) n (%)	AV7909 Alone Group 3 n (%)
Randomized (N)	NA	NA	70	NA	NA	71	69
Assigned (n)	45	25	NA	45	26	NA	NA
Not treated (n)	4 (8.9)	4 (16.0)	8 (11.4)	3 (6.7)	4 (15.4)	7 (9.9)	5 (7.2)
Treated (n)	41 (91.1)	21 (84.0)	62 (88.6)	42 (93.3)	22 (84.6)	64 (90.1)	64 (92.8)
Completed study treatment ¹ (received all study treatments)	30 (66.7)	21 (84.0)	51 (72.9)	39 (86.7)	19 (73.1)	58 (81.7)	61 (88.4)
Discontinued study	11 (24.4)	0	11 (15.7)	3 (6.7)	3 (11.5)	6 (8.5)	3 (4.3)
Received antibiotics only	3 (6.7)	0	3 (4.3)	1 (2.2)	2 (7.7)	3 (4.2)	NA
Received one vaccination	3 (6.7)	0	3 (4.3)	0	1 (3.8)	1 (1.4)	3 (4.3)
Received two vaccinations	5 (11.1)	0	5 (7.1)	2 (4.4)	0	2 (2.8)	0
Completed 12-month safety follow-up	33 (73.3)	21 (84.0)	54 (77.1)	39 (86.7)	20 (76.9)	59 (83.1)	61 (88.4)

Disposition	Cipro + AV7909 Group 1A n (%)	Cipro + AV7909 Group 1B n (%)	Cipro + AV7909 Group 1 (1A + 1B) n (%)	Doxy + AV7909 Group 2A n (%)	Doxy + AV7909 Group 2B n (%)	Doxy + AV7909 Group 2 (2A + 2B) n (%)	AV7909 Alone Group 3 n (%)
Primary reason for treatment discontinuation:	--	--	--	--	--	--	--
Adverse event	2 (4.4)	0	2 (2.9)	1 (2.2)	0	1 (1.4)	0
Death	0	0	0	0	0	0	0
Withdrawal by subject	6 (13.3)	0	6 (8.6)	1 (2.2)	1 (3.8)	2 (2.8)	1 (1.4)
Lost to follow-up	0	0	0	0	0	0	1 (1.4)
Physician decision	1 (2.2)	0	1 (1.4)	1 (2.2)	0	1 (1.4)	0
Other	2 (4.4)	0	2 (2.9)	0	0	0	1 (1.4)
Missing	0	0	0	0	2 (7.7)	2 (2.8)	0
Primary reason for study withdrawal:	--	--	--	--	--	--	--
Adverse event	1 (2.2)	0	1 (1.4)	0	0	0	0
Death	0	0	0	0	0	0	0
Lost to follow-up	0	0	0	1 (2.2)	2 (7.7)	3 (4.2)	1 (1.4)
Non-compliance with study drug	0	0	0	0	0	0	0
Physician decision	1 (2.2)	0	1 (1.4)	1 (2.2)	0	1 (1.4)	0
Withdrawal by subject	4 (8.9)	0	4 (5.7)	1 (2.2)	0	1 (1.4)	1 (1.4)
Other	1 (2.2)	0	1 (1.4)	0	0	0	0

N: number of subjects in the ITT Population; n = number of subjects; % = percentage based on number of randomized subjects
NA = not applicable.

Treatment groups: Group 1A=AV7909 + ciprofloxacin (with PK assessment); Group 1B=AV7909 + ciprofloxacin (without PK assessment); Group 2A=AV7909 + doxycycline (with PK assessment); Group 2B=AV7909 + doxycycline (without PK assessment); Group 3=AV7909

¹Subjects received two vaccinations with AV7909 and completed three courses of antibiotics as per protocol.

Ref: STN 125761/0, EBS.AVA.210, CSR, Table 12, pages 64-65 of 129, Figure 2, page 66 of 129. Source: Table 14.1.1.1; STN 125761/0, EBS.AVA.210, CSR Addendum 1, Table 2, pages 8-10 of 35; Source: Table 14.1.1.1a

There were 210 subjects who were randomized into EBS.AVA.210, with a similar number of subjects randomized to Group 1 (n=70; ciprofloxacin AV7909), Group 2 (n=71; doxycycline + AV7909), and Group 3 (n=69; AV7909 only). Subjects were randomized into four clinical study sites, with a higher number and proportion of subjects randomized to two study sites (US2002 and US2003). Of the 210 subjects randomized in the ITT Population, 48 subjects (22.9%) were randomized to study site US2001, 66 subjects (31.4%) were randomized to study site US2002, 86 subjects (41.0%) were randomized to study site US2003, and 10 subjects (4.8%) were randomized to study site US2004 (STN 125761/0. EBS.AVA.210, CSR, Table 14.1.3 (not shown), pages 122 of 565; Source 16.2.1.4).

Reviewer comment: Randomization of the ITT population by study site was not balanced; most of the subjects (72.4%) were enrolled at two study sites (US2002 and US2003). While the Applicant did not analyze PK or immunogenicity data by study site, no significant differences in PK or immunogenicity findings by study group were seen.

The proportion of subjects who completed all study treatments ranged from 66.7% (Group 1A: Ciprofloxacin + AV7909) to 88.4% (AV7909 alone); the mean proportion of subjects who completed all treatments across all study groups was 81% (n=170). There were 174 subjects (82.9%) who completed the 12-month safety follow-up. The primary reason for treatment

discontinuation and study withdrawal was ‘withdrawal by subject’ (n=9, 4.3% for treatment discontinuation and n=6, 2.9% for study withdrawal); one subject (0.5%) discontinued the study due to AEs.

Reviewer comment: *In general, subjects who were randomized to the antibiotic plus AV7909 groups had a somewhat lower completion rate of all treatments than subjects who received AV7909 alone; most likely due to the side effects of antimicrobials.*

The Applicant assessed whether the COVID-19 pandemic had an impact on the conduct of this study and determined that it did not have any significant effect. The Treatment Period had concluded in March 2020 at the start of the COVID-19 pandemic. The last day that the study drug was administered was on March 5, 2020 (Subject US(b) (6) [REDACTED]), which was before the World Health Organization announced the COVID-19 pandemic on March 11, 2020. The final Day 51 visit was on March 19, 2020. The study sites ensured that the in-clinic visits from March 11, 2020, to March 19, 2020, were in accordance with the applicable local pandemic measures. All ongoing subjects then entered the Safety Follow-up Period (i.e., via phone calls).

Reviewer comment: *The COVID-19 pandemic did not appear to have a significant effect on subject disposition, completion of treatment, or safety follow-up since the study had already initiated and was well underway by early 2020.*

A summary of the study’s analysis populations is provided in Table 19, below.

Table 19. EBS.AVA.210: Analysis Populations

Analysis Population	Cipro + AV7909 Group 1A n (%)	Cipro + AV7909 Group 1B n (%)	Cipro + AV7909 Group 1 (1A + 1B) n (%)	Doxy + AV7909 Group 2A n (%)	Doxy + AV7909 Group 2B n (%)	Doxy + AV7909 Group 2 (2A + 2B) n (%)	AV7909 Alone Group 3 n (%)	TOTAL n (%)
Intent-to-Treat (ITT) ¹	45 (100.0)	25 (100.0)	70 (100.0)	45 (100.0)	26 (100.0)	71 (100.0)	69 (100.0)	210 (100.0)
Safety ²	41 (91.1)	21 (84.0)	62 (93.3)	42 (93.3)	22 (84.6)	64 (90.1)	64 (92.8)	190 (90.5)
PK ³	25 (55.6)	NA	NA	31 (68.9)	NA	NA	NA	NA
Immunogenicity ⁴	28 (62.2)	19 (76.0)	47 (67.1)	36 (80.0)	14 (53.8)	50 (70.4)	52 (78.3)	151 (71.9)

n = number of subjects; % = percentage of subjects. NA: Not applicable.

Treatment groups: Group 1A = AV7909 + ciprofloxacin (with PK assessment); Group 1B = AV7909 + ciprofloxacin (without PK assessment); Group 2A = AV7909 + doxycycline (with PK assessment); Group 2B = AV7909 + doxycycline (without PK assessment); Group 3 = AV7909 only

¹The ITT Population included all randomized subjects. ²The Safety Population included all randomized subjects who received at least one dose of either antibiotic or AV7909. The PK³ and Immunogenicity⁴ Population included subjects who were randomized and met the criteria as specified in the protocol Section 10.3.

Ref: STN 125761/0, EBS.AVA.210, CSR, Table 14, page 69 of 129; Source: Table 14.1.1.2

The ITT Population comprised 210 (100.0%) randomized subjects. The Safety Population comprised 190 (90.5%) randomized subjects, with 151 (71.9%) of randomized subjects included in the Immunogenicity Population. The number of subjects in the PK population was lower, with 55.6% randomized subjects in the ciprofloxacin PK population and 68.9% of randomized subjects in the doxycycline PK population, respectively.

Reviewer comment: *The reason for the lower number of subjects in the treatment populations (Immunogenicity and PK) was due to exclusion of subjects from these analysis populations due to protocol deviations that precluded them from meeting the requisite Immunogenicity and PK Population criteria (subjects excluded from the PK Population and Immunogenicity Population are provided in Listings 16.2.3.1 and 16.2.3.2, respectively).*

Protocol deviations for all randomized subjects (ITT population) for the completed study (for the study duration of 12 months after the last vaccine dose) indicate that a total of 185 (88.1%) ITT subjects had at least one protocol deviation, with no significant difference in the incidence of protocol deviations across treatment groups (Group 1 (n=62, 88.6%); Group 2 (n=68, 95.8%), and Group 3 (n=55, 79.7%)).

Most subjects (170/210; 81.0%) had a minor protocol deviation and only two subjects (1.0%) had a critical protocol deviation, as described below.

- Subject US(b) (6) (Group 3): The subject was randomized into the study; however, was confirmed to meet exclusion criteria #5 (had a tattoo/scar/ birthmark or any other skin condition affecting the deltoid area that could interfere with injection site assessments) and was excluded from the Immunogenicity Population (Listing 16.2.5.3).
- Subject US(b) (6) (Group 1A): The subject's urine pregnancy test was not done at Day 8 (per clinical database). However, all pregnancy tests performed up through Day 51 were negative (Listing 16.2.8.3).

There were 105 (50.0%) subjects who had a major protocol deviation, with deviations primarily related to IP compliance (n=43 subjects total [20.5%]; n=21 [30.0%] in Group 1; n= 21 [29.6%] in Group 2; and n=1 subject [1.4%] in Group 3) followed by 'Other criteria,' which primarily included protocol deviations related to subject e-diary compliance (n=32 total [15.2%]), and 'Visit Schedule' Criteria (n=32 total [15.2%]).

Reviewer comment: *The majority of subjects had both major and minor protocol deviations. The impact of the critical protocol deviations described above on subject safety or data integrity were considered to be minimal by the Applicant. The clinical reviewer does agree that the critical protocol deviations cited above most likely did not affect the PK or immunogenicity results for EBS.AVA.210 in any significant manner. However, the clinical reviewer notes that many of the 'major' protocol deviations were related to treatment compliance (especially antimicrobial compliance) which could have possible effects on the PK data and/or immunogenicity results obtained in this study (see discussion of PK data in Section 6.2.11, below).*

Treatment Compliance

Compliance with antibiotic treatment (ciprofloxacin and doxycycline) is summarized in Table 20 below.

Table 20. EBS.AVA.210: Summary of Antibiotic Compliance (Safety Population)

Category	Cipro + AV7909 Group 1A N=41 n (%)	Cipro + AV7909 Group 1B N=21 n (%)	Cipro + AV7909 Group 1 (1A + 1B) N=62 n (%)	Doxy + AV7909 Group 2A N=42 n (%)	Doxy + AV7909 Group 2B N=22 n (%)	Doxy + AV7909 Group 2 (2A + 2B) N=64 n (%)
Number of subjects taking at least 50% of protocol-specified doses ¹	35 (85.4)	19 (90.5)	54 (87.1)	41 (97.6)	16 (72.7)	57 (89.1)
Number of subjects taking at least 5 of 7 in-clinic ciprofloxacin doses or 7 of 9 in-clinic doxycycline doses prior to each PK assessment ²	32 (78.0)	NA	NA	40 (95.2)	NA	NA

N = number of subjects in the Safety Population; n = number of subjects with medications; PK = pharmacokinetics.

Treatment groups: Group 1A = AV7909 + ciprofloxacin (with PK assessment); Group 1B = AV7909 + ciprofloxacin (without PK assessment); Group 2A = AV7909 + doxycycline (with PK assessment); Group 2B = AV7909 + doxycycline (without PK assessment).

¹All antibiotic doses administered in-clinic by staff and self-administered by subjects at home in the study.

²Number of subjects in Group 1A who received at least 5 of 7 in-clinic ciprofloxacin doses between Day 4 through the morning of Day 8 and between Day 31 through the morning of Day 35; or in Group 2A who received at least 7 of 9 in-clinic doxycycline doses between Day 2 through the morning of Day 8 and between Day 32 through the morning of Day 38.

Ref: STN 125761/0, EBS.AVA.210, CSR, Table 18, page 73 of 129; Source: Table 14.1.7.1

The majority of subjects were considered antibiotic compliant (defined PP as having taken at least 50% of the protocol-specified doses) with 54/62 (87.1%) subjects in Group 1 compliant with ciprofloxacin treatment and 57/64 (89.1%) of subjects in Group 2 compliant with doxycycline treatment. Antibiotic compliance, as summarized by e-diary (Tables 14.3.4.8.1 and 14.3.4.8.2) indicated that the majority of subjects (45/64, 70.3%) were compliant with the antibiotic e-diary completion (i.e., had >75% completion) (STN 125761/0, EBS.AVA.210, Table 14.3.4.8.1). Similarly, of the number of subjects who had at least one AV7909 vaccination (n=132/184; 71.7% total), most were compliant with the reactogenicity e-diary completion (i.e., had >75% completion) (Table 14.3.4.8.2).

Reviewer comment: *The compliance rate for antibiotic dosing was reasonable within the constraints of the prespecified definition of antibiotic ‘compliance,’ though not likely optimal in terms of subjects having had received all or most antibiotic doses. The Applicant’s criteria for compliance allowed subjects to miss a significant number of doses (up to 50%) and still be considered compliant with treatment. The significance of the antibiotic compliance rate observed in EBS.AVA.210 in terms of its impact on PK results is difficult to determine, though it is possible that an allowance to miss up to 50% of antibiotic doses may have contributed to the slightly lower AUC_{0-12h} observed for doxycycline. Compliance with AV7909 vaccination in the randomized subject population in EBS.AVA.210 appeared adequate, as most study subjects received two AV7909 vaccinations in accordance with the protocol (n=174, 91.6%), as summarized in Table 18 ‘Subject Disposition’ in Section 6.2.10.1.3 of the review memorandum.*

6.2.11 Efficacy Analyses

The primary efficacy endpoints for this vaccine-antibiotic interaction study were PK endpoints related to ciprofloxacin and doxycycline administration (see below). There were no primary immunogenicity endpoints defined in this study. Immunogenicity endpoints were defined as secondary endpoints.

6.2.11.1 Analyses of Primary Endpoint(s)

Primary Endpoints for Study EBS.AVA.210 were defined as:

- The Area Under the Curve from 0 to 12 hours (AUC_{0-12h}) and maximum concentration (C_{max}) for ciprofloxacin on Days 8 and 35, and
- The AUC_{0-12h} and C_{max} for doxycycline on Days 8 and 38.

Pharmacokinetic Results (Primary Analysis)

Ciprofloxacin Pharmacokinetic (PK) Results and Analysis

Ciprofloxacin PK parameters pre- and post-AV7909 vaccination for single dose and steady-state assessments indicate that geometric mean ciprofloxacin exposure parameters (AUC_{0-12h} and C_{max}) were generally comparable pre- and post-vaccination for single-dose and steady-state assessments: a 5% to 7% lower systemic exposure for single-dose ciprofloxacin and 2% to 3% lower systemic exposure ratio for steady-state was observed post-vaccination based on the

parameter ratios. Geometric mean ciprofloxacin $t_{1/2}$ estimates were comparable pre- and post-vaccination for single dose and steady-state assessments and ranged between 4.031 to 4.525 hours. The median ciprofloxacin T_{max} was also comparable and ranged between 1.000 and 1.430 hours. Differences between single and steady-state PK days were consistent with the predicted accumulation of ciprofloxacin based on the dosing regimen and observed $t_{1/2}$.

Results of the statistical comparison of the ciprofloxacin exposure parameters (AUC_{0-12h} and C_{max}) for the primary PK endpoints are presented in Table 21 below.

Table 21. EBS.AVA.210: Equivalence Test of Primary Pharmacokinetic (PK) Analysis for Ciprofloxacin (Pharmacokinetics (PK) Population)

Category	PK Parameter (unit)	Comparison (Post-vac vs. Pre-vac)	Number of Subjects Assigned for PK Assessment	PK Population	Number of Non-missing Pairs	Geometric Mean of Ratios (Post-vac/Pre-vac)	90% CI for Geometric Mean of Ratios
Primary PK endpoint	AUC_{0-12h} (h*ng/mL)	Day 35 vs Day 8	41	25	25	0.9764	(0.8895, 1.0718)
Primary PK endpoint	C_{max} (ng/mL)	Day 35 vs Day 8	41	25	25	0.9706	(0.8693, 1.0838)

PK = pharmacokinetic; Post-vac = post-vaccination; Pre-vac = pre-vaccination; CI = confidence interval

The equivalence testing was constructed using paired two one-sided t-tests (TOST) with natural log transformation of PK parameters. Results obtained from transformed analyses were back transformed by exponentiation for presentation of the point estimates and 90% CIs for geometric mean ratio of AUC_{0-12h} and C_{max} .

Ref: STN 125761/0, EBS.AVA.210, CSR, Table 21, page 81 of 129; Source: Table 14.2.1.1.4

For the primary PK steady-state ciprofloxacin PK endpoint, following administration of ciprofloxacin pre- (Day 8) and post-AV7909 vaccination (Day 35), the 90% CIs of the mean ratios of the steady-state AUC_{0-12h} and C_{max} were fully contained within the pre-defined equivalence criteria of [0.80, 1.25], thereby meeting the primary ciprofloxacin PK (steady-state) objective.

Doxycycline Pharmacokinetic (PK) Results and Analysis

Doxycycline PK parameters pre- and post-AV7909 vaccination for single-dose and steady-state assessments indicate that geometric mean doxycycline exposure parameters (AUC_{0-12h} and C_{max}) were comparable pre- and post-vaccination for single-dose doxycycline assessments but were approximately 8% to 10% lower post-vaccination compared to pre-vaccination for the steady-state doxycycline PK assessment based on parameter ratios.

Geometric mean doxycycline $t_{1/2}$ estimates were comparable pre- and post-vaccination for the single-dose assessment but were longer for steady-state assessments (approximately 9.8 hours versus 11.5 to 13.6 hours, respectively). The median doxycycline T_{max} was also comparable and ranged between 2.000 and 2.500 hours.

Results of the statistical comparison of the doxycycline exposure parameters (AUC_{0-12h} and C_{max}) for the primary PK endpoints are presented in Table 22 below.

Table 22. EBS.AVA.210: Equivalence Test of Primary Pharmacokinetic (PK) Analysis for Doxycycline (Pharmacokinetics (PK) Population)

Category	PK Parameter (unit)	Comparison (Post-vac. Vs. Pre-vac)	Number of Subjects Assigned for PK Assessment	PK Population	Number of Non-missing Pairs	Geometric Mean of Ratios (Post-vac/Pre-vac)	90% CI for Geometric Mean of Ratios
Primary PK endpoint	AUC _{0-12h} (h*ng/mL)	Day 38 vs Day 8	42	31	30	0.9173	(0.8187, 1.0278)
Primary PK endpoint	C _{max} (ng/mL)	Day 38 vs Day 8	42	31	31	0.8974	(0.7841, 1.0271)

CI = confidence interval; PK = pharmacokinetic; Post-vac. = post-vaccination; Pre-vac = pre-vaccination.

The equivalence testing was constructed using paired two one-sided t-tests (TOST) with natural log transformation of PK parameters. Results obtained from transformed analyses were back transformed by exponentiation for presentation of the point estimates and 90% CIs for geometric mean ratio of AUC_{0-12h} and C_{max}.

Ref: BLA STN 125761/0, CSR EBS.AVA.210, Table 24, page 87 of 129. Source: Table 14.2.1.2.4

For the primary PK steady-state doxycycline endpoint, following administration of doxycycline pre- (Day 8) and post-AV7909 vaccination (Day 38), the 90% CI of the mean ratio of steady-state AUC_{0-12h} [90% CI: 0.8187, 1.0278] was fully contained within the predefined equivalence criteria of [0.80, 1.25] with an approximately 8% lower geometric mean of the ratios. However, the LB of the 90% CI of the mean ratio of steady-state C_{max} [90% CI: 0.7841, 1.0271] was not within the predefined equivalence limits, thereby not meeting the primary PK doxycycline objective.

Reviewer comment: While results from the statistical analyses showed that the geometric mean value of the steady-state post-vaccine AUC_{0-12h} was approximately 8% lower than the pre-vaccine value and the LB of the 90% CI of the mean ratio of steady-state C_{max} [90% CI: 0.7841, 1.0271] was not within the predefined equivalence limits, the CDER PK consultant (Xiaohui (Tracey) Wei, Ph.D., Office of Clinical Pharmacology, CDER) noted that the post-vaccine C_{max} and C_{trough} levels were found exceeding the efficacious concentrations and MIC values determined from the rhesus monkey anthrax model⁸ (as referenced in the doxycycline USPI⁹). In addition, even though the upper bound of the 90% CI of the GMR for single-dose AUC_{0-12h} [90% CI: 0.8636, 1.2829] was above the predefined equivalence limits, doxycycline exposures (AUC_{0-12h}, C_{max}) determined from Study EBS.AVA.210 were found to be similar to the doxycycline exposures reported in the doxycycline label⁹ and in the published literature, per CDER's PK consultant. The PK consultant therefore concluded that the findings of steady-state and single-dose PK differences for doxycycline pre- versus post-AV7909 vaccine are not clinically relevant in a PEP setting where doxycycline would be administered with AV7909 vaccine.

6.2.11.2 Analyses of Secondary Endpoints

Secondary endpoints were defined as the following:

- The AUC_{0-12h} and C_{max} for ciprofloxacin on Days 4 and 31 and for doxycycline on Days 2 and 32, and
- The geometric mean TNA NF₅₀ values two weeks after the second vaccination (administered on Day 37 ±1 day).

Secondary PK Analysis for Ciprofloxacin

Results of the statistical comparison of the ciprofloxacin exposure parameters (AUC_{0-12h} and C_{max}) for the secondary PK endpoints are presented in Table 23 below.

Table 23. EBS.AVA.210: Equivalence Test of Secondary Pharmacokinetic (PK) Analysis for Ciprofloxacin (Pharmacokinetic (PK) Population)

Category	PK Parameter (unit)	Comparison (Post-vac. Vs Pre-vac)	Number of Subjects Assigned for PK Assessment	PK Population	Number of Non-missing Pairs	Geometric Mean of Ratios (Post-vac/ Pre-vac)	90% CI for Geometric Mean of Ratios
Secondary PK endpoint	AUC _{0-12h} (h*ng/mL)	Day 31 vs Day 4	41	25	24	0.9278	(0.7851, 1.0966)
Secondary PK endpoint	C _{max} (ng/mL)	Day 31 vs Day 4	41	25	24	0.9459	(0.7895, 1.1332)

PK = pharmacokinetic; Post-vac. = post-vaccination; Pre-vac = pre-vaccination; CI = confidence interval

The equivalence testing was constructed using paired two one-sided t-tests (TOST) with natural log transformation of PK parameters. Results obtained from transformed analyses were back transformed by exponentiation for presentation of the point estimates and 90% CIs for geometric mean ratio of AUC_{0-12h} and C_{max}.

Ref: STN 125761/0, CSR EBS.AVA.210, Table 21, page 81 of 129; Source: Table 14.2.1.1.4

For the secondary endpoints of ciprofloxacin AUC_{0-12h} and C_{max}, following single dose administration of ciprofloxacin on Day 4 and Day 31, the LBs of the 90% CIs of the mean ratios of AUC_{0-12h} [90% CI: 0.7851, 1.0966] and C_{max} [90% CI: 0.7895, 1.1332] were slightly below the predefined equivalence criteria of [0.80, 1.25]; hence, the secondary PK objective for single dose ciprofloxacin was not met.

Reviewer comment: While the LBs of the 90% CIs of the mean ratios of AUC_{0-12h} [90% CI: 0.7851, 1.0966] and C_{max} [90% CI: 0.7895, 1.1332] following single dose ciprofloxacin administration were slightly below the predefined equivalence criteria of [0.80, 1.25], CDER's PK consultant) noted that the pre- and post-vaccine ciprofloxacin exposures (AUC_{0-12h}, C_{max}) following single dose were similar to the ciprofloxacin exposures in human subjects as reported from ciprofloxacin label⁶ and exceeded the efficacious exposures and MIC values determined from the rhesus monkey anthrax model.^{7,8} The PK consultant therefore concluded that the findings of single-dose PK differences for ciprofloxacin pre- vs. post-AV7909 vaccine are not clinically relevant in a PEP setting where ciprofloxacin would be administered with AV7909 vaccine.

Secondary PK Analysis for Doxycycline

Results of the statistical comparison of the doxycycline exposure parameters (AUC_{0-12h} and C_{max}) for the secondary PK endpoints are presented in Table 24 below.

Table 24. EBS.AVA.210: Equivalence Test of Secondary Pharmacokinetic (PK) Analysis for Doxycycline (Pharmacokinetic (PK) Population)

Category	PK Parameter (unit)	Comparison (Post-vac. Vs Pre-vac)	Number of Subjects Assigned for PK Assessment	PK Population	Number of Non-missing Pairs	Geometric Mean of Ratios (Post-vac/ Pre-vac)	90% CI for Geometric Mean of Ratios
Secondary PK endpoint	AUC _{0-12h} (h*ng/mL)	Day 32 vs Day 2	42	31	26	1.0525	(0.8636, 1.2829)
Secondary PK endpoint	C _{max} (ng/mL)	Day 32 vs Day 2	42	31	26	1.0082	(0.8224, 1.2361)

PK = pharmacokinetic; Post-vac. = post-vaccination; Pre-vac = pre-vaccination; CI = confidence interval

The equivalence testing was constructed using paired two one-sided t-tests (TOST) with natural log transformation of PK parameters. Results obtained from transformed analyses were back transformed by exponentiation for presentation of the point estimates and 90% CIs for geometric mean ratio of AUC_{0-12h} and C_{max}.

Ref: STN 125761/0, CSR EBS.AVA.210, Table 24, page 87 of 129. Source: Table 14.2.1.2.4

The secondary doxycycline PK endpoint was evaluated and reported for information only. The 90% CI of the GMR for single-dose C_{max} [90% CI: 0.8224, 1.2361] was fully contained within the predefined equivalence criteria of [0.80 to 1.25], while the upper bound of the 90% CI of the GMR for single-dose AUC_{0-12h} [90% CI: 0.8636, 1.2829] was above the predefined equivalence limits.

Reviewer comment: Results from the statistical analyses conducted by the Applicant showed that the geometric mean values of doxycycline steady-state AUC_{0-12h} post-vaccination was approximately 8% lower than the pre-vaccine value; the LB of the 90% CI of the mean ratio of steady-state C_{max} [90% CI: 0.7841, 1.0271] was not within the predefined equivalence limits. However, the post-vaccine C_{max} and C_{trough} levels were found exceeding the efficacious concentrations and MIC values determined from the rhesus monkey anthrax model.⁸ In addition, even though the upper bound of the 90% CI of the GMR for single-dose AUC_{0-12h} [90% CI: 0.8636, 1.2829] was above the predefined equivalence limits, doxycycline exposures (AUC_{0-12h} , C_{max}) observed in Study EBS.AVA.210 were similar to exposures reported in the doxycycline label⁹ and in the published literature, per CDER's PK consultant and not deemed clinically relevant.

Immunogenicity Evaluation (Secondary Endpoint)

Immunogenicity analysis in EBS.AVA.210 assessed whether the immune response to AV7909 two weeks following the final dose of a two-dose vaccination series was affected by concomitant dosing with oral ciprofloxacin or doxycycline. Immunogenicity was assessed using TNA NF_{50} GMTs.

The non-inferiority test was constructed using the GMT ratio of TNA NF_{50} (Group 1/Group 3 or Group 2/Group 3) between the IP group with subjects that received both AV7909 and ciprofloxacin or doxycycline (Group 1 or 2) and the IP group with subjects that received AV7909 only (Group 3). The point estimate and the 95% CI for the GMT ratios were estimated using linear regression based on log 10 transformed TNA NF_{50} with equal variance. If the LB of the two-sided 95% CI of GMR was greater than the non-inferiority margin of 0.5, it was concluded that the immune response in the group who received AV7909 plus either ciprofloxacin or doxycycline was non-inferior to the group which received AV7909 alone and thus that neither ciprofloxacin nor doxycycline demonstrably affected the immunogenicity of AV7909.

Immunogenicity evaluation of Groups 1-3 is summarized in Table 25 below.

Table 25. EBS.AVA.210: Immunogenicity Evaluation as Assessed by TNA NF_{50} at Day 37 (Immunogenicity Population)

	Group 1 (Cipro + AV7909) N=47 n%	Group 2 (Doxy + AV7909) N=50 n%	Group 3 (AV7909 alone) N=54 n%	All N=151 n%
TNA NF_{50} at Day 37				
Mean (SD)	3.1 (5.4)	3.0 (5.2)	2.5 (3.6)	2.9 (4.7)
Median	1.8	1.8	1.5	1.8
Min, Max	<LLOQ, 36.9	0.27, 29.9	0.29, 19.4	<LLOQ, 36.9
GMT	1.8	1.8	1.6	1.7
95% CI	1.3, 2.4	1.4, 2.3	1.3, 2.0	1.5, 2.0

TNA NF_{50} = toxin-neutralizing antibody 50% neutralization factor. N = number of subjects in each treatment group in the Immunogenicity Population; n = number of valid immunogenicity outcome values; SD = standard deviation. GMT = geometric mean titer; CI = confidence interval

Treatment groups: Group 1 = AV7909 + ciprofloxacin; Group 2 = AV7909 + doxycycline; Group 3 = AV7909 only.

TNA NF_{50} LLOQ = 0.064. TNA NF_{50} values below the lower limit of quantitation (LLOQ) were imputed as 0.032 in the calculations of Mean/SD/GMT, which was half of the LLOQ of the assay.

Ref: STN 125761/0, CSR EBS.AVA.210, Table 25, page 91 of 129. Source: Table 14.2.2.1; Table 26, page 92 of 129; Source: 14.2.2.2.1; Table 28, page 92 of 129; Source: Table 14.2.2.2.2.

The TNA NF₅₀ values were below lower limit of quantitation (LLOQ) on Day 1 for all subjects in the Immunogenicity Population. Assessment of the immunogenicity of AV7909 two weeks after administration of the final AV7909 dose (of a two-dose vaccination) when concomitantly dosed with oral ciprofloxacin was performed using non-inferiority testing of the geometric mean TNA NF₅₀ between subjects who received AV7909 and ciprofloxacin (Group 1) and those who received AV7909 only (Group 3), as shown in Table 25 above.

As shown in Table 26 below, the LB of the two-sided 95% CI of GMR (0.78, 1.64 95% CI of Ratio) was greater than the non-inferiority margin of 0.5, indicating that the immune response in subjects who received AV7909 plus ciprofloxacin (Group 1) was non-inferior to the immune response in subjects who received AV7909 alone (Group 3). The GMT Ratio of Group 1/Group 3 was 1.13 (STN 125761/0, EBS.AVA.210, CSR, Table 26, page 92 of 129 Source: Table 14.2.2.2.1). Administration of ciprofloxacin thus did not appear to demonstrably affect the immunogenicity of AV7909.

Table 26. EBS.AVA.210: Non-Inferiority Test for Geometric TNA NF₅₀ between Treatment Groups with and without Ciprofloxacin or Doxycycline at Day 37 (Immunogenicity Population)

TNA NF ₅₀ at Day 37	GMT Ratio	95% CI of GMT Ratio
Group 1 (N=47)/Group 3 (N=54)	1.13	0.78, 1.64
Group 2 (N=50)/Group 3 (N=54)	1.14	0.81, 1.60

TNA NF₅₀ = toxin-neutralizing antibody 50% neutralization factor; GMT = geometric mean titer; CI = confidence interval
N = number of subjects in each treatment group in the Immunogenicity Population.

Treatment groups: Group 1 = AV7909 + ciprofloxacin; Group 2 = AV7909 + doxycycline; Group 3 = AV7909 only.

TNA NF₅₀ values below the lower limit of quantitation (LLOQ) were imputed as 0.032 in the calculations of Mean/SD/ GMT, which was half of the LLOQ of the assay.

The point estimate and 95% CI ratio were estimated using linear regression based on log₁₀ transformed TNA NF₅₀ with equal variance. If the lower bound of the 2-sided 95% CI of geometric mean ratio was > the non-inferiority margin on 0.5, the pre-specified non-inferiority success criterion was met.

Ref: STN 125761/0, EBS.AVA.210, CSR, Table 26, page 92 of 129; Source: 14.2.2.2.1; Table 28, page 92 of 129; Source: Table 14.2.2.2.2.

Similarly, assessment of the immunogenicity of AV7909 two weeks after administration of the final AV7909 dose (of a two-dose vaccination) when dosed concomitantly with oral doxycycline was performed using non-inferiority testing of the geometric mean TNA NF₅₀ between subjects who received AV7909 and doxycycline (Group 2) and those who received AV7909 only (Group 3).

The LB of the two-sided 95% CI of GMR (0.81, 1.60; 95% CI of ratio) was greater than the non-inferiority margin of 0.5, concluding that the immune response in subjects who received AV7909 plus doxycycline (Group 1) was non-inferior to the immune response in subjects who received AV7909 alone (Group 3) (data not shown; STN 125761/0, EBS.AVA.210, CSR, Table 28, page 93 of 129; Source: Table 14.2.2.2.2). Administration of doxycycline did not appear to demonstrably affect the immunogenicity of AV7909.

Reviewer comment: *Coadministration of ciprofloxacin or doxycycline with AV7909 did not appreciably affect the immune response to AV7909 vaccination, as measured by TNA NF₅₀ GMT pre- and post-vaccination. The CDER PK consultant recommended specific revisions to the USPI language in Sub-sections 7.1 and 14.2 based on the antimicrobial PK and*

AV7909 immunogenicity results. Details of the recommended language are provided in Section 11.5 of this clinical review memorandum.

6.2.11.3 Subpopulation Analyses

For immunogenicity analysis, TNA NF₅₀ values were summarized for each study group by: (1) age (18-30 and 31-45 years), and (2) sex (male, female). Please see the discussion of subgroup analysis under Section 6.2.11.5 below.

6.2.11.4 Dropouts and/or Discontinuations

The proportion of subjects who completed all study treatments ranged from 66.7% (Group 1A: Ciprofloxacin + AV7909) to 88.4% (AV7909 alone); the mean proportion of subjects who completed all treatments across all study groups was 81% (n=170). The subject withdrawal rate was somewhat higher for the AV7909 plus ciprofloxacin group. Subjects who withdrew from the study were not replaced; missing data were not imputed.

Reviewer comment: *The Applicant's handling of subject withdrawals and missing data was appropriate.*

6.2.11.5 Exploratory and Post Hoc Analyses

Exploratory analysis was performed using linear regression to evaluate the potential impact of imbalance in sex and age on the immunogenicity endpoints. There were no notable differences in geometric mean TNA NF₅₀ ratios and corresponding 95% CIs calculated using the primary (unadjusted) and exploratory (adjusted for site, sex, and age) analyses, indicating that there was no significant impact of sex and age on immunogenicity responses.

Reviewer comment: *Evaluation for interference effects of ciprofloxacin and doxycycline on the immune response to AV7909 showed no appreciable effect of age or sex on the immune response to AV7909 (i.e., post-vaccination geometric mean TNA NF₅₀ ratios and corresponding 95% CIs).*

6.2.12 Safety Analyses

6.2.12.1 Methods

Safety monitoring procedures (assessment) for EBS.AVA.210 were previously summarized under Section 6.2.7 Surveillance/Monitoring. Safety analyses and summaries were based on the Safety Population (i.e., all randomized subjects who received at least one dose of antibiotic or AV7909); subjects were included according to the treatment they received. Summary safety tables and listings included all safety data collected up to Day 51, with additional safety data (SAEs and AESIs) collected for 12 months after administration of the second AV7909 vaccine dose (provided in the EBS.AVA.210 CSR Addendum 1). The safety tables provided in this review include all safety data to 12 months post-final vaccination and include revised safety data (AEs, local injection site, and systemic reactogenicity events) submitted under STNs 125761/0/27, 125761/0/31, and 125761/0/38.

As a result of the updates to the CE and AE datasets (STN 125761/0/27) and the corrected ADFACE and ADAE datasets (STN 125761/0/31 and 125761/0/38), e-diary symptoms that continued past Day 7 post-vaccination were reclassified as AEs in the revised safety database.

Only those solicited events recorded up to Day 7 were included in the e-diary database. In addition, any solicited symptom post-vaccination that was (1) serious (i.e., a solicited reaction confirmed by the investigator to be a Grade 4, or a Grade 3 that upon the investigator's assessment met any of the SAE criteria); (2) resulted in discontinuation of study product or withdrawal from the study; or (3) remained unresolved for 14 days or more, was recorded as an AE. Safety data presented in this review of EBS.AVA.210 incorporate the revised safety dataset findings, where applicable (i.e., e-diary and TEAE discussion).

Reviewer comment: *In summary, revision of the safety datasets for EBS.AVA.210 resulted in the AEs of reactogenicity occurring through Day 7 post-vaccination being removed from the AE dataset unless they were serious. Reactogenicity events that were collected from the e-diary and continued past Day 7 post-vaccination but not beyond Day 14, were added to the AE datasets. Therefore, some events were removed from the AE summaries and added to the solicited reactogenicity data summaries, while some longer lasting solicited events were removed from the reactogenicity summaries and added to the AE summaries.*

Extent of Exposure

Most study subjects received two AV7909 vaccinations in accordance with the protocol (n=174, 91.6%). The proportion of subjects who had two vaccinations, as PP, were similar across all treatment groups (Group 1: ciprofloxacin + AV7909 (n=55, 88.7%), Group 2: doxycycline + AV7909 (n=59, 92.2%), and Group 3: AV7909 only (n=60 subjects, 93.8%)). Extent of exposure to antibiotics (ciprofloxacin and doxycycline) was previously discussed in Section 6.2.10.1.3 Subject Disposition.

6.2.12.2 Overview of Adverse Events

Local and Systemic Reactogenicity

Local and systemic reactogenicity (i.e., solicited AEs related to vaccination) were assessed by subject e-diary and during in-clinic evaluation. Subjects were instructed to complete a post-vaccination diary for at least seven days after administration of each vaccine dose, until all solicited local and systemic symptoms resolved after each vaccination. AEs were also collected from in-clinic exams performed immediately prior to, and at least 30 minutes following, each injection.

Local and Systemic Reactogenicity Assessed by E-Diary

E-diary reactogenicity data were summarized by treatment group, with the number and percentage of subjects who reported each local reactogenicity symptom (warmth, tenderness, itching, pain, AML, redness, induration, swelling, and bruising) and systemic reaction (tiredness/fatigue, muscle ache, headache, and fever) by severity grade (0=no reaction, 1=mild, 2=moderate, 3=severe and 4=life-threatening), per FDA's Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials.³³

A summary of local and systemic reactogenicity as assessed by subject e-diary for each vaccination, and for each of the study treatment groups is summarized in Tables 27 and 28 below, respectively.

Table 27. EBS.AVA.210: Local Reactogenicity Assessed by E-Diary Post-Vaccination with AV7909 (Safety Population)

Injection Site Reaction	AV7909 + Ciprofloxacin (Group 1) 1st Vaccination (N=59) n, n%	AV7909 + Doxycycline (Group 2) 1st Vaccination (N=61) n, n%	AV7909 Alone (Group 3) 1st Vaccination (N=64) n, n%	AV7909 + Cipro (Group 1) 2nd Vaccination (N=56) n, n%	AV7909 + Doxycycline (Group 2) 2nd Vaccination (N=60) n, n%	AV7909 Alone (Group 3) 2nd Vaccination (N=61) n, n%
Any injection site reaction	--	--	--	--	--	--
Any grade	42 (77.8)	49 (86.0)	52 (82.5)	36 (66.7)	40 (70.2)	49 (86.0)
≥ Grade 3	1 (1.9)	1 (1.8)	4 (6.3)	0	1 (1.8)	2 (3.5)
Tenderness	--	--	--	--	--	--
Any grade	38 (70.4)	46 (80.7)	48 (76.2)	35 (64.8)	38 (66.7)	46 (80.7)
≥ Grade 3	0	1 (1.8)	0	0	0	0
Pain	--	--	--	--	--	--
Any grade	35 (64.8)	30 (55.6)	50 (79.4)	42 (73.7)	35 (61.4)	45 (78.9)
≥ Grade 3	0	0	1 (1.6)	0	1 (1.8)	1 (1.8)
AML ²	--	--	--	--	--	--
Any grade	22 (40.7)	27 (47.4)	30 (47.6)	23 (42.6)	25 (43.9)	30 (52.6)
≥ Grade 3	0	0	0	0	0	0
Warmth	--	--	--	--	--	--
Any grade	18 (33.3)	18 (31.6)	26 (41.3)	20 (37.0)	20 (35.1)	22 (38.6)
≥ Grade 3	0	0	0	0	1 (1.8)	0
Induration	--	--	--	--	--	--
Any grade	11 (20.4)	19 (33.3)	14 (22.2)	16 (29.6)	20 (35.1)	19 (33.3)
≥ Grade 3	0	0	2 (3.2)	0	0	1 (1.8)
Bruise	--	--	--	--	--	--
Any grade	1 (1.9)	3 (5.3)	3 (4.8)	5 (9.3)	5 (8.8)	9 (15.8)
≥ Grade 3	0	0	1 (1.6)	0	1 (1.8)	0
Itching	--	--	--	--	--	--
Any grade	6 (11.1)	9 (16.7)	10 (15.9)	13 (22.8)	16 (28.1)	14 (24.6)
≥ Grade 3	0	0	0	0	1 (1.8)	0
Swelling	--	--	--	--	--	--
Any grade	4 (7.4)	12 (21.1)	9 (14.3)	13 (24.1)	12 (21.1)	12 (21.1)
≥ Grade 3	1 (1.9)	0	2 (3.2)	0	0	1 (1.8)

Injection Site Reaction	AV7909 + Ciprofloxacin (Group 1) 1st Vaccination (N=59) n, n%	AV7909 + Doxycycline (Group 2) 1st Vaccination (N=61) n, n%	AV7909 Alone (Group 3) 1st Vaccination (N=64) n, n%	AV7909 + Cipro (Group 1) 2nd Vaccination (N=56) n, n%	AV7909 + Doxycycline (Group 2) 2nd Vaccination (N=60) n, n%	AV7909 Alone (Group 3) 2nd Vaccination (N=61) n, n%
Erythema/redness	--	--	--	--	--	--
Any grade	5 (9.3)	9 (15.8)	10 (15.9)	11 (20.4)	13 (22.8)	13 (22.8)
≥ Grade 3	1 (1.9)	0	2 (3.2)	0	0	1 (1.8)

Treatment groups: Group 1 = AV7909 + ciprofloxacin; Group 2 = AV7909 + doxycycline; Group 3 = AV7909 only.

N = Number of subjects in the Safety Population who received first, second, and any vaccination; n = Number of subjects with a reaction. % = n/(n with non-missing Grade)*100

¹For each vaccination site, each subject was counted only once under the most severe intensity rating.

²AML: Arm motion limitation

Ref: STN 125761/0/31, EBS.AVA.210, Final Safety Lock, CSR Addendum 1, Table 14.3.4.9.1b, pages 1-10. Source: Listing 16.2.8.8.1

Table 28. EBS.AVA.210: Systemic Reactogenicity Assessed by E-Diary Post-Vaccination with AV7909 (Safety Population)

Injection Site Reaction	AV7909 + Ciprofloxacin (Group 1) 1st Vaccination (N=59) n, n%	AV7909 + Doxycycline (Group 2) 1st Vaccination (N=61) n, n%	AV7909 Alone (Group 3) 1st Vaccination (N=64) n, n%	AV7909 + Cipro (Group 1) 2nd Vaccination (N=61) n, n%	AV7909 + Doxycycline (Group 2) 2nd Vaccination (N=60) n, n%	AV7909 Alone (Group 3) 2nd Vaccination (N=61) n, n%
Any systemic reaction	--	--	--	--	--	--
Any grade	32 (59.3)	43 (75.4)	42 (66.7)	29 (53.7)	40 (70.2)	41 (71.9)
≥ Grade 3	0	1 (1.8)	4 (6.3)	1 (1.9)	0	5 (8.8)
Tiredness/fatigue	--	--	--	--	--	--
Any grade	21 (38.9)	29 (50.9)	29 (46.0)	21 (38.9)	28 (49.1)	30 (52.6)
≥ Grade 3	0	1 (1.8)	2 (3.2)	1 (1.9)	0	2 (3.5)
Headache	--	--	--	--	--	--
Any grade	18 (33.3)	20 (35.1)	25 (39.7)	13 (24.1)	25 (43.9)	25 (43.9)
≥ Grade 3	0	0	4 (6.3)	1 (1.9)	0	3 (5.3)
Myalgia	--	--	--	--	--	--
Any grade	26 (48.1)	35 (61.4)	39 (61.9)	27 (50.0)	30 (52.6)	36 (63.2)
≥ Grade 3	0	0	2 (3.2)	1 (1.9)	0	1 (1.8)

Injection Site Reaction	AV7909 + Ciprofloxacin (Group 1) 1st Vaccination (N=59) n, n%	AV7909 + Doxycycline (Group 2) 1st Vaccination (N=61) n, n%	AV7909 Alone (Group 3) 1st Vaccination (N=64) n, n%	AV7909 + Cipro (Group 1) 2nd Vaccination (N=61) n, n%	AV7909 + Doxycycline (Group 2) 2nd Vaccination (N=60) n, n%	AV7909 Alone (Group 3) 2nd Vaccination (N=61) n, n%
Fever ²	--	--	--	--	--	--
Any grade	1 (1.9)	0	3 (4.8)	1 (1.9)	0	3 (5.3)
≥ Grade 3	0	0	1 (1.6)	0	0	2 (3.5)

Treatment groups: Group 1 = AV7909 + ciprofloxacin; Group 2 = AV7909 + doxycycline; Group 3 = AV7909 only.

N = Number of subjects in the Safety Population who received first, second, and any vaccination; n = Number of subjects with a reaction. % = n/ (n with non-missing Grade) *100.

¹For each vaccination site, each subject was counted only once under the most severe intensity rating.

²The toxicity grade for fever is derived based on the combination of oral temperature subjects self-reported in the e-diary and any additional oral temperature readings provided by the subject. Toxicity grades were set to NULL for temperature less than 90°F or greater than 110°F.

Ref: STN 125761/0/31, EBS.AVA.210, Final Safety Lock, CSR Addendum 1, Table 14.3.4.9.2b, pages 1-6. Source: Listing 16.2.8.

The most commonly reported local reactogenicity symptoms (in order of frequency) comprised tenderness, pain, AML, and warmth; with a slightly higher frequency reported in the AV7909 only group (Group 3).

For all treatment groups (combined), most subjects reported either Grade 1 (75/184; 40.8%) or Grade 2 (71/184; 38.6%) severity of local injection site reactions (redness or swelling at the injection site) in the e-diaries after any vaccination. A higher proportion of subjects in Group 3 (5/64 [7.8%]) reported Grade 3 injection site reactions after any vaccination, compared with Group 1 (1/59 [1.7%]) and Group 2 (1/61 [1.6%]). There were no reports of Grade 4 injection site reactions. In general, there was no meaningful difference in the proportion of subjects reporting any injection site reactions collected from e-diaries after the first vaccination, the second vaccination, or after any vaccination, respectively, for Group 1 subjects (42/59 [71.2%], 36/56 [64.3%], and 47/59 [79.7%]), Group 2 subjects (49/61 [80.3%], 40/60 [66.7%], and 51/61 [83.6%]), or Group 3 subjects (52/64 [81.3%], 49/61 [80.3%], and 55/64 [85.9%] subjects).

Reviewer comment: *In general, there was no significant across-treatment difference in the incidence of injection site reactions reported in the e-diaries. Injection site reactions were generally similar in frequency after the 1st and 2nd vaccinations for the AV7909 alone group (Group 3), but slightly lower in frequency after the 2nd vaccination for the two, antibiotic plus AV7909 groups (Groups 2 and 3). Most injection site reactions were reported as Grade 1 or 2 severity.*

For systemic reactions assessed by e-diary, the most frequently reported symptoms were myalgia, fatigue, followed by headache. There were no elevated temperatures recorded post-vaccination per e-diary, with the exception of one subject in Group 1 who reported Grade 1 fever after the second AV7909 vaccination (see Table 28, above). Systemic reactions collected from e-diaries were more frequently reported in Groups 2 and 3 compared to Group 1, but this difference was not significantly higher numerically. Overall, most subjects reported systemic reactions in e-diaries after any vaccination as Grade 1 or Grade 2 severity. There were no Grade 4 systemic reactions.

In general, there was no meaningful difference in the proportion of subjects with any solicited systemic reactions after the first vaccination, the second vaccination, or after any vaccination, respectively for Group 1 subjects (32/59 [54.2%], 29/56 [51.8%], and 40/59 [67.8%]), Group 2 subjects (43/61 [70.5%], 40/60 [66.7%], and 49/61 [80.3%]), or Group 3 subjects (42/64 [65.6%], 41/61 [67.2%], and 48/64 [75.0%]).

Local and Systemic Reactogenicity Assessed In-Clinic

In-clinic reactogenicity data were collected in a similar manner as the e-diary reactogenicity findings, except for fever (oral temperature), which was collected pre- and 30 minutes post-vaccination and presented as a vital sign observation. In-clinic systemic reactogenicity symptoms comprised fatigue, headache, muscle ache, and fever and were recorded at the same time (pre- and 30 minutes post-vaccination) as the local reactogenicity symptoms.

The incidence of in-clinic injection site reactions was low (n= 9/184 or 4.9% after the first vaccination for all treatment groups combined), with most subjects reporting no injection site reactions (i.e., Grade 0). While the most frequently reported in-clinic reactogenicity symptoms across treatment groups were warmth followed by tenderness and pain; these were reported in <10% of study subjects and all were Grade 1 in severity. There was no meaningful difference in the proportion of subjects with any injection site reactions collected in clinic after the first

vaccination, the second vaccination, or after any vaccination in either Group 1, 2, or 3. There was also no remarkable across-treatment difference in the incidence of injection site reactions.

In-clinic systemic reactions were only reported in 3 (1.6%) subjects overall; and all were Grade 1 in severity. Systemic reactogenicity symptoms were reported in-clinic in one Group 3 subject (muscle ache) and in one Group 2 subject (fatigue) after the first AV7909 dose and one Group 3 subject (muscle ache) after the second AV7909 dose; the vast majority of subjects reported no systemic reactogenicity symptoms in-clinic.

Reviewer comment: *Local and systemic reactogenicity in Study EBS.AVA.210 were recorded as being relatively infrequent, based on in-clinic assessments. All reported local and systemic reactions in-clinic were mild in severity (Grade 1).*

Treatment Emergent Adverse Events (TEAEs)

TEAEs that occurred in ≥5% of subjects in the Safety Population using the revised safety datasets are presented in Table 29 below and include injection site pain (10.0%), injection site induration (7.4%), vaccination complication (7.4%) and musculoskeletal procedural complication (6.3%). For systemic reactogenicity, headache was the most frequently reported unsolicited AE (n=8/190; 4.2%) (STN 125761/0, EBS.AVA.210, CSR Addendum 2, Table 3, pages 15-16 of 32).

The proportion of subjects who had at least one TEAE was higher in Group 1 (n=32/62; 51.6%) than in Group 2 (n=29/64; 45.3) or Group 3 (n=28/64; 43.8) (STN 125761/0/38, EBS.AVA.210, EBS.AVA.210, CSR Addendum 2, Table 14.3.1.a).

There were no meaningful differences across the three study groups regarding the specific TEAEs reported, although the proportion of subjects with injection site induration was lower in Group 1 (n=2/62, 3.2%) than in Group 2 (n=7/64, 10.9%) or Group 3 (n=5/64, 7.8%).

Most of the events were either Grade 1 (28.9%) or Grade 2 (10.0%) in severity. Grade 3 TEAEs occurred in 8.9% (17/190) of subjects overall; and were reported in slightly higher rates in the antimicrobial coadministration groups: Group 1 (n=6/62; 11.3%) and Group 2 (n=6/64; 9.4%), compared to the AV7909 alone group: Group 3 (n=4/64; 6.3%) (STN 125761/0/38, EBS.AVA.210, CSR Addendum 2, Table 2, page 24 of 32; Source: Table 14.3.1.1a). There were no Grade 4 TEAEs reported.

Table 29. Study EBS.AVA.210: Treatment-Emergent Adverse Events (TEAEs) Occurring in ≥ 5% of Subjects in AV7909 or BioThrax Groups by SOC and PT (Safety Population)

MedDRA System Organ Class Preferred Term	Group 1 cipro+AV7909 (N=62) n (%)	Group 2 doxy+AV7909 (N=64) n (%)	Group 3 AV7909 alone (N=64) n (%)	Total (N=190) n (%)
All TEAEs	32 (51.6)	29 (45.3)	28 (43.8)	89 (46.8)
Gastrointestinal Disorder	--	--	--	--
Nausea	2 (3.2)	4 (6.3)	1 (1.6)	7 (3.7)
Vomiting	1 (1.6)	4 (6.3)	1 (1.6)	6 (3.2)
General Disorders and Administration Site Conditions	--	--	--	--
Injection site pain	6 (9.7)	5 (7.8)	8 (12.5)	19 (10.0)
Injection site induration	2 (3.2)	7 (10.9)	5 (7.8)	14 (7.4)
Injection site movement impairment	3 (4.8)	2 (3.1)	4 (6.3)	9 (4.7)

MedDRA System Organ Class Preferred Term	Group 1 cipro+AV7909 (N=62) n (%)	Group 2 doxy+AV7909 (N=64) n (%)	Group 3 AV7909 alone (N=64) n (%)	Total (N=190) n (%)
Injury, Poisoning and Procedural Complications	--	--	--	--
Vaccination complication	3 (4.8)	4 (6.3)	7 (10.9)	14 (7.4)
Musculoskeletal procedural complication	4 (6.5)	4 (6.3)	4 (6.3)	12 (6.3)
Procedural headache	2 (3.2)	4 (6.3)	2 (3.1)	8 (4.2)

TEAE=Treatment-emergent adverse event; N = Number of subjects in the Safety Population; n = Number of subjects with AEs within each group exposed to the treatment (incidence); % = n/N*100; cipro = ciprofloxacin; doxy = doxycycline; SOC = System organ class; PT = Preferred term

MedDRA = Medical Dictionary for Regulatory Activities; AEs were coded according to MedDRA version 22.0.

Each subject was counted once for each applicable category. The SOCs were sorted in alphabetical order and PTs within each SOC were sorted in descending order of percentage in the AV7909 column.

Ref: STN 125761/0/31, EBS AVA.210, CSR Addendum 2, Table 3, pages 15-16 of 32. Source: Table 14.3.1.1a and Table 14.3.1.8a; 125761/0/38, Table 14.3.1.2a, pages 1-22; Source: Listing 16.2.7.1a

The cumulative proportion of subjects for the completed study who had AEs leading to study drug discontinuation (n=3, 1.6%) and study withdrawal (n=1, 0.5%) was low.

Antibiotic-related TEAEs in Groups 1 (related to ciprofloxacin) or 2 (related to doxycycline) occurred in 9.7 % and 12.5% of subjects, respectively. The most frequent antibiotic-related AEs occurred in Group 2 (related to doxycycline) and comprised nausea (n=5/64; 7.8%) and vomiting (n=4/64; 6.3%).

Reviewer comment: TEAEs were reported at a slightly higher frequency in the AV7909 group (Group 3), over the combined antibiotic plus AV7909 groups (Groups 1 and 2). While the Applicant did not offer an explanation or rationale to explain this finding, the addition of antibiotic to AV7909 administration likely led to subjects' greater focus and reporting of TEAEs more commonly associated with antibiotic use (e.g., nausea and vomiting were reported more frequently in these groups) than TEAEs more commonly associated with vaccination (e.g., injection site reactions).

6.2.12.3 Deaths

No deaths were reported in EBS.AVA.210 at the time data cut-off on Day 51 up to the final day of safety follow-up at Day 337 (STN 125761/0, EBS.AVA.210, CSR Addendum, Section 12.3 and 12.3.1, Source: Table 14.3.2.1.1).

6.2.12.4 Nonfatal Serious Adverse Events

There were no SAEs reported up to Day 51 of the study (STN 125761/0, EBS.AVA.210, Day 51 CSR, Section 12.3.3.2, Source: Table 14.3.1.9 and Table 14.3.1.10).

Two subjects were reported to have an SAE after the Day 51 cutoff, though neither was related to AV7909 vaccination (STN 125761/0, EBS.AVA.210, CSR Addendum 1, Section 12.3.1; Source: Table 14.3.2.1.2a):

- Subject US(b) (6) A 34-year-old White female in Group 1A developed a Grade 3 SAE of viral gastroenteritis 333 days after the first administration of AV7909 and 318 days after the first administration of ciprofloxacin. This SAE was not considered related to treatment and resolved.

- Subject US(b) (6) : A 41-year-old Black female in Group 3 with a past medical history of ectopic pregnancy, missed abortion, who was gravida six; and a surgical history of caesarian section and premature separation of placenta; developed a Grade 2 SAE of uterine prolapse 85 days after the first administration of AV7909. The uterine prolapse was treated surgically; the SAE resolved. The SAE was not considered related to treatment.

6.2.12.5 Adverse Events of Special Interest (AESIs)

There were no reported AESIs from time of data cut-off at Day 51 through the last day of follow-up on Day 337 (End of Study) (STN 125761/0, EBS.AVA.210, CSR Addendum 1, Section 12.3.2).

6.2.12.6 Clinical Test Results

No clinically meaningful changes from baseline were observed for hematology and serum chemistry and there were no important treatment group differences noted in the mean change from baseline laboratory values over time (STN 125761/0, EBS.AVA.210, CSR, Section 12.4.2). No clinically significant findings were noted from urinalysis, VS measurements, or PE findings.

6.2.12.7 Dropouts and/or Discontinuations

One subject had an AE leading to study withdrawal (STN 125761/0, EBS.AVA.210, CSR, Table 37, page 101 of 129; Listing 16.2.7.3). Subject US(b) (6) reported a Grade 2 AE of ankle fracture that was unrelated to IP. Administration with ciprofloxacin was discontinued and subject was withdrawn from the study due to this event. At the Applicant's last report, this AE was resolving.

One subject (US(b) (6) in Group 1A had a Grade 1 AE of pharyngitis that led to discontinuation of AV7909, although the AE was deemed unrelated to IP administration (Table 14.3.1.13). At the Applicant's last report, this event was resolved (STN 125761/0, EBS.AVA.210, CSR, Table 37, page 101 of 129; Listing 16.2.7.3).

Two subjects had AEs leading to antibiotic discontinuation. One subject in Group 1 (Subject US(b) (6) as described above) had an AE of ankle fracture that led to discontinuation of ciprofloxacin administration. Subject US(b) (6) had Grade 1 AEs of lower abdominal pain (possibly related to IP), vomiting ('definitely related' to study drug), and headache (possibly related to IP) that led to discontinuation of doxycycline administration. At the Applicant's last report, all three events were resolving (STN 125761/0, EBS.AVA.210, CSR, Table 37, page 101 of 129; Listing 16.2.7.3).

6.2.13 Study Summary and Conclusions

Pharmacokinetics of Ciprofloxacin and Doxycycline

EBS.AVA.210 was designed as a Phase 2, AV7909-antimicrobial interaction study which examined the effects of vaccination on ciprofloxacin and doxycycline PK and conversely the effect of antimicrobial administration on AV7909 immunogenicity in healthy adults 18-45 years of age.

The primary PK endpoint for each antibiotic was met if the 90% CI of the geometric mean of the within-subject ratios were contained entirely within the equivalence bounds of [0.8, 1.25] for both

AUC_{0-12h} and C_{max} at steady state. The secondary PK endpoint for each antibiotic was met if the 90% CIs of the geometric mean of the within-subject ratios.

IM administration of a 2-dose regimen of AV7909 had no statistically significant effect on the steady-state of ciprofloxacin (primary PK endpoint), based on pre-specified PK equivalence criteria. For the secondary endpoints of single dose ciprofloxacin PK measurements AUC_{0-12h} and C_{max} , predefined equivalence criteria were not met, as they were slightly below the predefined equivalence criteria of [0.80, 1.25].

IM administration of a 2-dose regimen of AV7909 resulted in 8-10% lower steady-state exposure of doxycycline. Based on pre-specified PK equivalence criteria for steady-state doxycycline AUC_{0-12h} and C_{max} , the primary PK endpoint for doxycycline was not met.

For the secondary PK endpoint for single dose doxycycline, the first equivalence criterion was met (the 90% CI for the mean ratio for C_{max} [90% CI: 0.82, 1.24] was fully contained within the predefined equivalence criteria of [0.80, 1.25]), but the second equivalence criterion was not met (the upper bound of the 90% CI of the mean ratio for AUC_{0-12h} [90% CI: 0.86, 1.28] was slightly above the predefined upper equivalence limit of 1.25).

Despite the PK findings for single dose ciprofloxacin administration and for steady state and single dose doxycycline administration these PK findings were not considered clinically significant. CDER's PK consultant indicated that the pre- and post-vaccine ciprofloxacin exposures (AUC_{0-12h} , C_{max}) following single dose ciprofloxacin were similar to ciprofloxacin exposures in human subjects reported in the ciprofloxacin label⁶ and exceeded the efficacious exposures and MIC values determined from the rhesus monkey anthrax model that was used to support licensure of ciprofloxacin for PEP against anthrax.⁸

Similarly, the doxycycline exposures (AUC_{0-12h} , C_{max}) determined from Study EBS.AVA.210 were similar to the doxycycline exposures reported in the doxycycline label⁹ and in the published literature.⁸ The PK consultant concluded that the findings of steady-state and single-dose PK differences for doxycycline pre- vs. post-AV7909 vaccine are not clinically relevant in a PEP setting where doxycycline would be administered with AV7909 vaccine.

Immunogenicity

As the LB of the two-sided 95% CI of GMR was greater than the non-inferiority margin of 0.5, it was concluded that the immune response in subjects who received AV7909 plus ciprofloxacin (Group 1) or in those who received AV7909 plus doxycycline (Group 2) was non-inferior to the immune response in subjects who received AV7909 alone (Group 3); the addition of ciprofloxacin or doxycycline did not significantly change the immune response to AV7909. There were no notable differences in geometric mean TNA NF_{50} ratios and corresponding 95% CIs calculated using the primary (unadjusted) and exploratory (adjusted for site, sex, and age) analyses, indicating that there was no significant impact of sex and age on AV7909 immunogenicity responses. The study met the prospectively defined success criteria for the secondary immunogenicity analyses, with a conclusion that administration of ciprofloxacin and doxycycline did not demonstrably affect the immunogenicity of AV7909.

Safety

Submitted safety data indicated that the PEP schedule of AV7909 was well tolerated and had an overall acceptable safety profile. Safety follow-up to Day 337/End of Study by telephone follow-up calls did not identify any new safety findings. The most common AEs reported were those related to AV7909 post-vaccination local and systemic reactogenicity (i.e., tenderness,

pain, and myalgia) and were generally mild to moderate in severity (Grade 1 or 2). A slightly higher proportion of subjects in the AV7909 only group (Group 3) reported Grade 3 injection site and systemic reactions as compared to the AV7909 groups that also received ciprofloxacin or doxycycline (Groups 1 and 2). There were no Grade 4 solicited injection site or systemic reactions.

TEAEs that occurred in $\geq 5\%$ of subjects in the Safety Population included injection site pain (10.0%), injection site induration (7.4%), vaccination complication (7.4%) and musculoskeletal procedural complication (6.3%). No significant changes in laboratory parameters or PEs were reported. During the 12-month safety follow-up period after administration of the final dose of AV7909, no deaths, SAEs related to AV7909 vaccination, or AESIs were reported, consistent with the safety findings previously reported up to the final clinic visit on Day 51. Updates to the safety datasets submitted by the Applicant under STN 125761/0/27 (November 18, 2022) and STN 125761/0/38 (February 28, 2023), respectively, resulted in no changes to AEs related to abnormal clinical laboratory results (hematology, serum chemistry, urinalysis), AEs related to vital sign or physical exam findings, SAEs, AESIs, or pregnancy/fetal outcomes. Changes in local and systemic reactogenicity by e-diary and TEAE frequencies were minimally affected by this revised safety database and did not change the safety profile of AV7909 or safety conclusions regarding this vaccine.

In summary, no apparent safety concerns were observed for AV7909 administered alone or in combination with ciprofloxacin or doxycycline throughout the entire study period. The two-dose AV7909 vaccination co-administered IM with either ciprofloxacin or doxycycline or without antibiotics demonstrated an acceptable safety profile for the study duration (1 year post last vaccine dose), as assessed under study EBS.AVA.210.

7. INTEGRATED OVERVIEW OF EFFICACY

While studies EBS.AVA.201, -208, -210 and -212 all evaluated the 'to-be-marketed' formulation of AV7909, an ISE was not performed, because these studies were designed to assess immunogenicity at different time points, and in some cases (e.g., EBS.AVA.201 and -210) evaluated different immunogenicity endpoints. In addition, an ISE of AV7909 studies was not performed given that this application is an animal rule "efficacy" approval and animal studies had been completed and reviewed for efficacy under the BLA for BioThrax (and all components of those studies met our data standards for animal rule studies), and animal studies for AV7909 demonstrated effectiveness of AV7909 against inhalational anthrax disease after exposure to *B. anthracis*. The pivotal clinical study submitted to this BLA demonstrated noninferiority of the immunogenicity of AV7909 to BioThrax which established the link to the animal rule approval of BioThrax, in addition to establishing an immunobridge to animal studies conducted with AV7909.

Please refer to Sections 6.1, 6.2 and 9.2 for discussion of the individual studies.

8. INTEGRATED OVERVIEW OF SAFETY

8.1 Safety Assessment Methods

The original ISS was submitted with Part 2 of the rolling Biologics License Application (BLA) (125761/0) on April 20, 2022. Two addenda to the ISS were submitted under STNs 125761/0/27 (November 18, 2022) and 125761/0/38 (February 28, 2022) to address dataset discrepancy issues identified during the review process by the BLA review team.

The Applicant's ISS addendum under STN 125671/0/27 included the following:

1. Addition of BioThrax (AVA) comparator arm safety data for EBS.AVA.201, -208 and -212,
2. Updates to safety information for the CE and AE datasets (AEs, local injection site and systemic reactogenicity events) for studies EBS.AVA.210 and -212, and
3. Exclusion of study site US1027 safety data in Study EBS.AVA.212 due to GCP issues identified at this site as part of the Applicant's sensitivity analysis.

An ISS addendum for select tables was submitted under STN 125761/0/38 to provide revised source tables for solicited reactions in the ISS (and respectively, for studies EBS.AVA.210 and -212 that were part of the ISS) to exclude subjects who failed to provide any e-diary data from the denominator of the respective dose and to address ADAE inaccuracies identified by the statistical reviewer.

Reviewer comment: *Safety data reviewed for the ISS comprise safety data provided in ISS Addendum 3 under STN 125761/0/27, with additional revisions incorporated in the ISS review from corrected ISS tables submitted STN 125761/0/38.*

Evaluation of safety data in the ISS was conducted using a tiered approach. Safety data in the ISS comprised separate analyses for the AV7909 (b) (4) formulation/dosing regimen (**Tier 1 analysis**) and all AV7909 formulations/dosing regimens assessed in clinical studies (**Tier 2 analysis**). Safety data for the BioThrax group were included as active comparator data (different routes, dosing regimens) in ISS Addendum 3 (see discussion above).

Reviewer comment: *Analyses based on data from all subjects who received at least one dose of AV7909 using any formulation or dosing schedule from the four studies in the "Tier 2" analysis were included in the ISS review as supplementary information (ISS Addendum Sections 4.2 and 5.3).*

Lastly, methods for the ISS required realignment of safety assessment time points following vaccination across all studies, since the day of randomization differed in the earlier studies EBS.AVA.201 and -208 (where the day of randomization was Day 0) from that of the later studies (EBS.AVA.210 and -212, where the day of randomization was Day 1). In addition, the day of vaccinations also differed across studies (e.g., in Study EBS.AVA.210, randomizations occurred on Day 1, but vaccinations were administered on Days 8 and 23), requiring a re-adjustment to account for different study days when the same schedule of AV7909 was administered (two weeks apart).

8.2 Safety Database

8.2.1 Studies/Clinical Trials Used to Evaluate Safety

The Applicant conducted four clinical studies; each of these assessed the (b) (4) formulation of AV7909 (AVA 0.5 mL plus 0.25 mg CpG 7909) in at least one of the study groups. The respective dosing regimens and number of subjects randomized in these four studies are summarized in Table 30 below.

Table 30. Integrated Summary of Safety (ISS): Summary of AV7909 Clinical Studies

Study	Dosing Regimen	Subjects Treated ¹ , per Group	Any AV7909: Total Treated
EBS.AVA.210: Phase 2, Vaccine-Drug Interaction Study	2 IM injections at 0 and 2 weeks Arm 1: AV7909 + ciprofloxacin Arm 2: AV7909 + doxycycline Arm 3: AV7909 alone	62 64 64	190
EBS.AVA.212 ² : Phase 3, Safety, Immunogenicity, Lot-to-lot Consistency Study	3 injections at 0, 2 and 4 weeks ³ Arm 1: AV909 Lot 1 Arm 2: AV909 Lot 2 Arm 3: AV909 Lot 3 Arm 4: BioThrax SC (AVA 0.5 mL)	1050 1053 1048 533	3151
EBS.AVA.201: Phase 1 Immunogenicity and Safety	2 IM injections 2 weeks apart (Day 0, 14): Arm 1: BioThrax (0.5 mL) Arm 2: 0.5 mL AVA + 0.5 mg CpG 7909 Arm 3: 0.5 mL AVA + 0.25 mg CpG 7909 (AV7909) ⁴ Arm 4: 0.25 mL AVA + 0.5 mg CpG 7909 Arm 5: 0.25 mL AVA + 0.25 mg CpG 7909 Arm 6: Saline (Placebo)	18 18 17 19 18 15	90
EBS.AVA.208: Phase 2, Immunogenicity and Safety	3 IM injections at 0, 2, 4 weeks: Arm 1: AV7909, AV7909, saline Arm 2: AV7909, saline, AV7909 Arm 3: AV7909, AV7909, AV7909 Arm 4: ½ dose AV7909 ⁵ , ½ dose AV7909, ½ dose AV7909 Arm 5: BioThrax, BioThrax, BioThrax	44 34 23 44 23	145

¹Treated indicates subject received at least one dose of study treatment.

²Study Site US1027 excluded from subjects treated/group for Study EBS.AVA.212.

³AV7909 given IM on Weeks 0 and 2; placebo control given IM on Week 4.

⁴AV7909 (full dose): 0.5 mL (0.5 mL AVA + 0.25 mg CpG 7909).

⁵Half-dose (1/2 dose) AV7909: 0.25 mL (0.25 mL AVA + 0.125 mg CpG 7909); Full BioThrax (AVA) dose: 0.5 mL.

Ref: Reviewer generated table compiled from: STN 125761/0, Section 2.7.6., STN 125761/0, EBS.AVA.201, CSR, Table 7, page 50 of 597, Source: Table 14.1.1; STN 125761/0, EBS.AVA.208, CSR, Table 8, pages 77-78 or 166, Source: Table 14.1.1; STN 125761/0, EBS.AVA.210, CSR, Table 12, pages 64-65 of 129, Source: Table 14.1.1.1.; STN 125761/0, EBS.AVA.210, CSR Addendum 1, Table 2, pages 8-10 of 129; CSR Addendum 2, Section 10.1; STN 125761/0/27, CSR Addendum 2, Table 2, pages 8-10 of 35, Source: Table 14.1.1.1a; STN 125761/0, EBS.AVA.212, CSR Addendum 2, Section 11.1., Table 3, page 18 of 74, Source: Table 14.1.1.2b; STN 125761/0/27, CSR Addendum 3, Section 11.1, page of 57.

Study V011 was a Phase 1, randomized (1:1:1), double-blind, active-controlled (BioThrax) parallel group study which assessed 0.5 mL AV7909 (administered as 0.5 mL AVA plus 0.25 mg CpG 7909, consisted of an admixture of CpG plus AVA) vs. 1 mg CpG 7909 alone and vs. 0.5 mL BioThrax given as three IM injections at Day 0, 14, and 28. The study was conducted by a different manufacturer (b) (4) using AVA that was administered as a separate injection apart from the CpG 7909 adjuvant. Because the vaccine formulation evaluated in V011 differed from the (b) (4) AV7909 formulation, clinical data from V0011 was not included in the ISS for STN 125761/0.

The ISS for this BLA therefore comprises integrated safety data for Studies EBS.AVA.201, -208, -210, and -212.

8.2.2 Overall Exposure, Demographics of Pooled Safety Populations

A summary of AV7909 and BioThrax exposure from the ISS is summarized in Table 31 below.

Table 31. Integrated Summary of Safety: AV7909 and BioThrax Exposure

Vaccinations Administered	Pooled AV7909 (Tier 1) (N=3276) n (%)	Pooled AV7909 (Tier 2) (N= 3552) n (%)	EBS.AVA.21 2 AV7909 Groups 1-3 (N=3151) n (%)	EBS.AVA.212 BioThrax (N=533) n (%)	EBS.AVA.208 BioThrax (N=23) n (%)	EBS.AVA.201 BioThrax (N=18) n (%)
Received 1 dose of study vaccine only	259 (7.9)	270 (7.6)	253 (8.0)	40 (7.5)	1 (4.3)	2 (11.1)
Received 2 doses of study vaccine	3017 (92.1)	3221 (90.7)	2898 (92.0)	21 (3.9)	0	16 (88.9)
Received 3 doses of study vaccine	NA	61 (1.7)	NA	472 (88.6)	22 (95.7)	NA

AV7909 Groups: In EBS.AVA.201, Group 2 = AVA 0.5mL + CpG 7909 0.5mg; Group 3 = AVA 0.5mL + CpG 7909 0.25mg; Group 4 = AVA 0.25mL + CpG 7909 0.5mg; Group 5 = AVA 0.25mL + CpG 7909 0.25mg. In EBS.AVA.208, Group 1 = AV7909/AV7909/Placebo; Group 2 = AV7909/Placebo/ AV7909; Group 3= Three doses AV7909; Group 4 = Three half-doses AV7909. In EBS.AVA.210, Group 1 = AV7909 + ciprofloxacin; Group 2= AV7909+doxycycline; Group 3 = AV7909 only. In EBS.AVA.212, Group 1 = AV7909 Lot 1; Group 2 = AV7909 Lot 2; Group 3 = AV7909 Lot 3. BioThrax Groups: EBS.AVA.201, Group 1; EBS.AVA.208, Group 5; EBS.AVA.212, Group 4.
N = Number of subjects in the safety set; n = Number of subjects within the category; % = n/N*100. NA – Data was not collected.
Ref: STN 125761/0/27, ISS Addendum 3, Table 11.1.1.3a

The majority of AV7909 vaccinated subjects (>90%) received both doses of AV7909. Similarly, for BioThrax vaccinated subjects, most subjects (>89%) received the three doses, per the licensed PEP schedule.

Subject demographic results for the Tier 1 and Tier 2 pooled populations, the pivotal Study EBS.AVA.212 and the respective BioThrax groups (where applicable) for each individual study are summarized in Table 32 below.

Table 32. ISS: Subject Demographics (Safety Population¹)

Demographic/ Category	Pooled AV7909 (Tier 1) N=3276 n (%)	Pooled AV7909 (Tier 2) N=3552 n (%)	EBS.AVA.2 12 AV7909 Groups 1-3 N=3151 n (%)	EBS.AVA.212 BioThrax N=533 n (%)	EBS.AVA.208 BioThrax N=23 n (%)	EBS.AVA.201 BioThrax N=18 n (%)
Age (Years)	--	--	--	--	--	--
Mean (SD)	39.0 (12.9)	38.5 (12.8)	39.2 (13.0)	38.7 (12.4)	32.5 (10.4)	31.1 (9.3)
Median	38.0	37.0	38.0	38.0	30.0	30.5
Min, Max	18, 65	18, 65	18, 65	18, 64	18, 48	18, 47
Sex, n (%)	--	--	--	--	--	--
Female	1895 (57.8)	2052 (57.8)	1826 (57.9)	293 (55.0)	11 (47.8)	9 (50.0)
FOCBP	1325 (40.4)	1423 (40.1)	1284 (40.7)	220 (41.3)	0	8 (44.4)
Male	1381 (42.2)	1500 (42.2)	1325 (42.1)	240 (45.0)	12 (52.2)	9 (50.0)
Race Category, n (%)	--	--	--	--	--	--
White	2553 (77.9)	2766 (77.9)	2452 (77.8)	416 (78.0)	21 (91.3)	15 (83.3)
Black/African American	561 (17.1)	617 (17.4)	541 (17.2)	88 (16.5)	2 (8.7)	2 (11.1)
Other/More than one race	162 (4.9)	169 (4.8)	158 (5.0)	29 (5.4)	0	1 (5.6)
Ethnicity, n (%)	--	--	--	--	--	--
Not Latino/ Hispanic	2747 (83.9)	2981 (83.9)	2646 (84.0)	425 (79.7)	19 (82.6)	14 (77.8)
Hispanic/ Latino	493 (15.0)	534 (15.0)	469 (14.9)	98 (18.4)	4 (17.4)	4 (22.2)
Not Reported	36 (1.1)	37 (1.0)	36 (1.1)	10 (1.9)	0	0

¹Safety Population excludes site US1027 for Study EBS.AVA.212.

N = Number of subjects in the safety set; n = Number of subjects within the category; % = n/N*100; Max =Maximum; Min =Minimum; SD = Standard deviation

Ref: STN 125761/0/27, ISS Addendum, Table 11.1.1.2a., pages 1-2.

Subject demographics for the pooled AV7909 populations (Tier 1 and 2 groups) were similar to that of AV7909 vaccinated subjects enrolled in the pivotal Study EBS.AVA.212, and to the BioThrax subjects enrolled in studies EBS.AVA.201, -208 and -212. Most subjects were White, with a slightly higher proportion of female subjects and subjects 31-50 years of age.

Subject baseline characteristics were likewise similar across the pooled AV7909 study groups (Tier 1 and 2) compared to AV7909 subjects in EBS.AVA.212 and compared to BioThrax vaccinated subjects across studies.

Reviewer comment: *There were no significant demographic or baseline population differences across studies or treatment arms that were likely to impact the integrated safety results or conclusions regarding AV7909.*

8.2.3 Categorization of Adverse Events

Different versions of MedDRA dictionary were used in coding of AEs in the four studies. To ensure coding consistency in the pooled database for ISS, AE data were converted to MedDRA dictionary version 22.0.

8.3 Caveats Introduced by Pooling of Data Across Studies/Clinical Trials

The pooling strategy and analysis groups for the ISS, as based on a tiered analysis comprised the two tiers and six groups total, as summarized in Table 33 below.

Table 33. ISS: Study Analysis Groups

ISS Group Number	ISS Group Name	Study Groups Included
1 (Tier 1)	Pooled AV7909 with 'to-be-marketed' formulation/regimen	EBS.AVA.201: Group 3
1 (Tier 1)	Pooled AV7909 with 'to-be-marketed' formulation/regimen	EBS.AVA.208: Group 1
1 (Tier 1)	Pooled AV7909 with 'to-be-marketed' formulation/regimen	EBS.AVA.210: Group 3
1 (Tier 1)	Pooled AV7909 with 'to-be-marketed' formulation/regimen	EBS.AVA.212: Groups 1-3
2 (Tier 2)	Pooled AV7909 with all formulations/regimens	EBS.AVA.201: Groups 2-5
2 (Tier 2)	Pooled AV7909 with all formulations/regimens	EBS.AVA.208: Group 1-4
2 (Tier 2)	Pooled AV7909 with all formulations/regimens	EBS.AVA.210: Groups 1-3
2 (Tier 2)	Pooled AV7909 with all formulations/regimens	EBS.AVA.212: Groups 1-3
3	EBS.AVA.212: Groups 1-3	Group 1: AV7909 Lot 1 at Day 1, 15; Placebo at Day 29
3	EBS.AVA.212: Groups 1-3	Group 2: AV7909 Lot 2 at Day 1, 15; Placebo at Day 29
3	EBS.AVA.212: Groups 1-3	Group 3: AV7909 Lot 3 at Day 1, 15; Placebo at Day 29
4	EBS.AVA.212: Group 4 BioThrax	Group 4: BioThrax SC at Day 1, 15, and 29
5	EBS.AVA.208: Group 5	Group 5: BioThrax IM at Day 1, 15, and 29
6	EBS.AVA.201: Group 1	Group 1: BioThrax IM at Day 1 and 15

For Groups 3-6, the safety population comprised all subjects for each respective treatment who received at least one dose. Ref: STN 125761/0/27, ISS Addendum, Table 6, page 34 of 111.

The ISS assessment was performed without Study EBS.AVA.212 site US1027 safety data, using revised CE and AE datasets (both submitted under 125761/0/27) and corrected ADAE datasets submitted under 12761/0/38. All analyses in the ISS were based on the Safety

Population (Safety Set), which included all randomized subjects who received at least one dose of study vaccine. The baseline value was defined as the last non-missing value prior to the first dose of study vaccine. Safety data in the ISS are presented as descriptive statistics displaying the incidence of events and the proportion of subjects with events.

The clinical reviewer focused on TEAEs, deaths, SAEs, and AESIs for the ISS review. A combined review of local and systemic reactogenicity across the four clinical studies was not performed by the clinical reviewer.

Reviewer comment: *The Applicant included pooling of solicited injection site reactions and solicited systemic reactions that combined e-diary with in-clinic reactogenicity assessments across studies as part of the reactogenicity pooling strategy. The clinical reviewer did not consider pooling of reactogenicity data across the four clinical studies appropriate for the purpose of the ISS because the individual studies used different methodologies to assess solicited local and systemic reactogenicity.*

TEAEs in the four studies are included in the pooled analyses. TEAEs are defined as AEs that presented after the first dose of AV7909 vaccination or any AE already present that worsened in either frequency or intensity following treatment. AEs with onset prior to the first vaccination but after the initiation of ciprofloxacin or doxycycline in Study EBS.AVA.210 are not included in the pooled database for this ISS.

Subgroup analysis of safety data (incidence of TEAEs and SAE) for Tier 1 (pooled AV7909 with ‘to-be-marketed’ regimen/formulation) and Tier 2 (pooled AV7909 with all regimens/formulations) was performed per the following demographic factors: age group (18-30, 31-50, and 51-65 years of age), sex (male, female), and race (White, Black or African American, and Other).

8.4 Safety Results

8.4.1 Deaths

Deaths were monitored for up to one year following the last vaccination. A total of seven deaths were reported across the four AV7909 clinical studies (one in Study EBS.AVA.201 and six in Study EBS.AVA.212) for the Tier 2 analysis. All deaths except one (Subject (b) (6) in Study EBS.AVA.201) occurred in subjects who were administered AV7909 with the ‘to-be-marketed’ regimen/formulation (Table 34, below).

Table 34. ISS: Deaths Reported Across All Clinical Studies

Study	Subject Number	Treatment Group/Test Product	Age (years)/ Sex/Race ¹	Interval Between Last Vaccine Dose and Event	Preferred Term (MedDRA v22.0)	Causality
EBS.AVA.201	(b) (6)	BioThrax	42/M/W	329 days	Road traffic accident	Unrelated
EBS.AVA.212	U (b) (6)	Group 1 / AV7909	52/F/W	Approximately 2 months	Toxicity to various agents (heroin)	Unrelated
EBS.AVA.212	U (b) (6)	Group 1 / AV7909	30/F/B	Approximately 8.5 months	Death (of unknown cause)	Unrelated
EBS.AVA.212	U (b) (6)	Group 2 / AV7909	25/M/W	Approximately 2 months	Completed suicide	Unrelated

Study	Subject Number	Treatment Group/Test Product	Age (years)/ Sex/Race ¹	Interval Between Last Vaccine Dose and Event	Preferred Term (MedDRA v22.0)	Causality
EBS.AVA.212	U (b) (6)	Group 2 / AV7909	24/M/O	78 days	Completed suicide	Unrelated
EBS.AVA.212	U (b) (6)	Group 3 / AV7909	55/M/W	Approximately 8 months	Overdose	Unrelated
EBS.AVA.212	U (b) (6)	Group 3 / AV7909	49/M/W	1 day	Toxicity to various agents ²	Unrelated

Abbreviations: EBS.AVA.212, Group 1 = AV7909 Lot 1; Group 2 = AV7909 Lot 2; Group 3 = AV7909 Lot 3.

¹Sex: M = Male, F = Female; Race: W = White, B = Black or African American, O = Other.

²Mitragynine is a primary alkaloid derived from the tropical tree of the same name. It is purported to have psychoactive effects and is commonly known as Kratom to recreational users.

Ref: STN 125761/0/27, ISS, Table 13, page 61 of 111; Source: Listing 11.2.1.

All deaths were determined to be unrelated to AV7909 by the study PIs. In all cases where the cause of death was reported (6/7 deaths), they were due to factors external to the involvement of subjects in AV7909 clinical studies. In the only event of death of unknown cause in subject US(b) (6) (EBS.AVA.212), there was no temporal association between the time of vaccination and time of death. This death occurred in circumstances external to Study EBS.AVA.212 and was deemed unrelated to AV7909. The one death reported in a BioThrax vaccinated subject enrolled in Study EBS.AVA.201 was due to extraneous circumstances (motor vehicle accident) and was deemed unrelated to vaccination.

8.4.2 Nonfatal Serious Adverse Events

SAEs were monitored for up to one year following the last vaccination. The proportion of AV7909 vaccinated subjects in the Tier 1 analysis (N=3424) who reported any SAE was low (1.81% in the pooled safety population [95% CI: 1.39, 2.32]); with a total of 77 events reported in 62 subjects (1.8%) (STN 125761/0, ISS Addendum, Source: Table 11.1.5.1 and Table 11.1.5.3). Most of the SAEs reported (76 events in 61 subjects (1.8%)) were not related to AV7909 vaccination. One event (acute cholecystitis in Subject US(b) (6) who received AV7909 Lot 1 in Study EBS.AVA.212; <0.1%) was deemed related to AV7909 by the PI. However, the DSMB and Applicant deemed the event unrelated to vaccination due to the subject's pre-existing history of, and evidence of active gallstone disease on ultrasound.

The proportion of SAEs reported in AV7909 vaccinated subjects was similar in the Tier 2 analysis (79 events in 64 subjects; 1.73%; [95% CI: 1.33, 2.20]) to that in Tier 1.

Of reported SAEs, no pattern or prevalence was seen per MedDRA SOC or PT classification (STN 125761/0, ISS Addendum, Source: Table 11.1.5.1); with a generally even distribution of events across all SOC categories. None of the reported SAEs by SOC and PT occurred in more than 0.3% and 0.2% of subjects, respectively within the pooled safety population by Tier 1 or 2 analysis.

Reviewer comment: *A slightly higher incidence of SAEs were reported for the SOC category of 'Injury, Poisoning and Procedural Complications' (13 reports in 12 subjects (0.4%)) but no discrete pattern in SAEs was seen in this SOC category.*

Subgroup analysis of SAEs by demographics in the Tier 1 and Tier 2 analyses showed slightly increased SAE reporting with age for both tiers (data not shown) STN125761/0, ISS Addendum, Table 11.1.5.2.1, pages 1-5).

Reviewer comment: *There was no similarity in SOC distribution for the SAEs between age subgroups; therefore, it is unlikely that there was a causal association of SAEs with AV7909 vaccination across age groups.*

The sex distribution of SAEs was similar between the two groups, with female subjects having a similar rate of SAEs (2.0%) as male subjects (1.6%). When considered by race, similar proportions of subjects reported SAEs across all groups; 1.9% of Black or African American subjects (n=11), 1.8% for subjects of both White (n=48) and Other (n=3). There is no evidence from this data that one race was more predisposed to SAEs following administration of two doses of AV7909.

Reviewer comment: *Based on the observed distribution of SAEs between and within subgroups, there is no evidence that any of the intrinsic factors would predispose subjects to the occurrence of SAEs, or occurrence of SAEs within a particular organ or body system following vaccination with AV7909.*

8.4.3 Study Dropouts/Discontinuations

Subject disposition for AV7909 vaccinated subjects in the Tier 1 and 2 analyses of the ISS is presented in Table 35 below.

Table 35. ISS: Subject Disposition¹

Disposition	Pooled AV7909 To-Be-Marketed Formulation/Regimen (Tier 1) N (%)	Pooled AV7909 All Formulations/Regimens (Tier 2) N (%)	EBS.AVA.212 AV7909 Groups 1-3 N (%)	EBS.AVA.212 BioThrax N (%)	EBS.AVA.208 BioThrax N (%)	EBS.AVA.201 BioThrax N (%)
Treated with vaccine (Safety Set, N)	3276	3552	3151	533	23	18
Vaccination completed	2846 (86.9)	3107 (87.5)	2731 (86.7)	472 (88.6)	22 (95.7)	16 (88.9)
Vaccination discontinued	430 (13.1)	445 (12.5)	420 (13.3)	61 (11.4)	1 (4.3)	2 (11.1)
Adverse event	102 (3.1)	105 (3.0)	99 (3.1)	15 (2.8)	0	0
Lost to follow-up	19 (0.6)	21 (0.6)	18 (0.6)	4 (0.8)	0	0
Withdrawal by subject	66 (2.0)	69 (1.9)	65 (2.1)	4 (0.8)	1 (4.3)	0
Death	1 (<0.1)	1 (<0.1)	1 (<0.1)	0	0	0
Other ²	240 (7.3)	243 (6.8)	237 (7.5)	38 (7.1)	0	0
No reason provided on eCRF	1 (<0.1)	5 (0.1)	0	0	0	2 (11.1)
Physician decision	1 (<0.1)	1 (<0.1)	0	0	0	0
Study completed	3067 (93.6)	3329 (93.7)	2948 (93.6)	512 (96.1)	21 (91.3)	15 (83.3)
Study withdrawal	209 (6.4)	223 (6.3)	203 (6.4)	21 (3.9)	2 (8.7)	3 (16.7)
Adverse event	2 (<0.1)	2 (<0.1)	1 (<0.1)	0	0	1 (5.6)
Lost to follow-up	141 (4.3)	146 (4.1)	138 (4.4)	17 (3.2)	1 (4.3)	1 (5.6)
Physician decision	4 (0.1)	4 (0.1)	4 (0.1)	0	0	0

Disposition	Pooled AV7909 To-Be-Marketed Formulation/Regimen (Tier 1) N (%)	Pooled AV7909 All Formulations/Regimens (Tier 2) N (%)	EBS.AVA.212 AV7909 Groups 1-3 N (%)	EBS.AVA.212 BioThrax N (%)	EBS.AVA.208 BioThrax N (%)	EBS.AVA.201 BioThrax N (%)
Subject/ consent withdrawal	49 (1.5)	56 (1.6)	48 (1.5)	4 (0.8)	1 (4.3)	1 (5.6)
Death	6 (0.2)	6 (0.2)	6 (0.2)	0	0	0
Other	7 (0.2)	9 (0.3)	6 (0.2)	0	0	0

N = Number of subjects in each treatment arm in the Safety Population % = Percentage of subjects based on the Safety Population (% = n/N*100)

¹Subject disposition based on revised safety datasets that exclude Site US1027 from Study EBS.AVA.212.

²'Other' reason for treatment discontinuation defined as 'Visit would have been out of treatment window.'

Ref: STN 125761/0/27, ISS Addendum 3, Table 11.1.1.1a

Subject disposition results for the ISS were not significantly different from that of the pivotal study EBS.AVA.212 and indicate that the most subjects received all vaccinations and completed all study visits for their respective clinical studies. Approximately 13% of subjects in both the Tier 1 and 2 analyses did not complete all vaccinations, most commonly a result of the study visit being outside of the treatment window (i.e., 'Other' category for vaccination discontinuation), followed vaccine discontinuation due to an AE (approximately 3% of subjects). The overall subject study withdrawal rate across clinical studies was also low (approximately 6.4%), with most study withdrawal cases due to lost to follow-up, and only a very small percentage of subjects withdrawing from the study due to an AE (<0.1%).

Reviewer comment: *Subject withdrawal from vaccination was reasonably low, with study withdrawal rates lower—most likely due to each study’s design and aim to continue assessing subjects who discontinued vaccination for safety evaluation follow-up for the entire study’s duration. AEs were not a significant reason for incompleteness of the vaccination series or for study withdrawal. Subject disposition results in the ISS confirmed similar subject disposition findings previously summarized for the pivotal study EBS.AVA.212, as these results were primarily carried by the large subject number contributed to the ISS by Study EBS.AVA.212.*

8.4.4 Common Adverse Events

Treatment-Emergent Adverse Events (TEAEs)

TEAEs were defined in the ISS as AEs that “occurred or worsened following the first vaccination regardless of causal relationship to AV7909”. All TEAEs from the four pooled clinical trials (which occurred following vaccination) through the last clinic visit were recorded and classified according to MedDRA terminology.

An overall summary of the number and frequency of TEAEs, as categorized by relatedness to vaccination, by severity Grade 3 or higher, and TEAEs that resulted in vaccine discontinuation or study withdrawal, is summarized in Table 36 below. Also included in this table, for a comparison basis, are the number and frequency of deaths, SAEs, and AESIs for each respective ISS study tier/group.

Table 36. ISS: Overall Summary of Treatment-Emergent Adverse Events (TEAEs; Safety Population¹)

Category	Pooled AV7909 (Tier 1) N=3276 n (%)	Pooled AV7909 (Tier 2) N=3552 n (%)	EBS.AVA.212 AV7909 Groups 1-3 N=3151 n (%)	EBS.AVA.212 BioThrax N=533 n (%)	EBS.AVA.208 BioThrax N=23 n (%)	EBS.AVA.201 BioThrax N=18 n (%)
TEAEs	1294 (39.5)	1478 (41.6)	1216 (38.6)	251 (47.1)	15 (65.2)	16 (88.9)
TEAEs related to treatment	487 (14.9)	575 (16.2)	453 (14.4)	169 (31.7)	2 (8.7)	15 (83.3)
Grade 3 or higher TEAEs	213 (6.5)	239 (6.7)	204 (6.5)	29 (5.4)	2 (8.7)	3 (16.7)
Grade 3 or higher TEAEs related to treatment	44 (1.3)	51 (1.4)	41 (1.3)	8 (1.5)	0 (0)	2 (11.1)
TEAEs from time of 1st vaccination to last clinic visit	1037 (31.7)	1215 (34.2)	961 (30.5)	221 (41.5)	14 (60.9)	16 (88.9)
SAEs	62 (1.9)	64 (1.8)	58 (1.8)	4 (0.8)	1 (4.3)	0 (0)
SAEs related to treatment	1 (0.03)	1 (0.03)	1 (0.03)	1 (0.2)	0 (0)	0 (0)
Deaths	6 (0.2)	7 (0.2)	6 (0.2)	0 (0)	0 (0)	0 (0)
Deaths related to treatment	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
TEAEs leading to treatment discontinuation	75 (2.3)	81 (2.3) 79 (2.2)	72 (2.3) 70 (2.2)	14 (2.6) 13 (2.4)	0 (0)	1 (5.6)
TEAEs leading to study withdrawal	2 (0.06)	2 (0.06)	1 (0.03)	0 (0)	0 (0)	1 (5.6)
AESIs	15 (0.5)	15 (0.4)	15 (0.5)	2 (0.4)	0 (0)	0 (0)
AESIs related to treatment	3 (0.09)	3 (0.08)	3 (0.10)	1 (0.2)	0 (0)	0 (0)

¹Safety Population excludes site US1027 from Study EBS.AVA.212

N = Number of vaccinated subjects; n = Number of subjects with TEAEs; % = n/N*100; TEAE = Treatment-emergent adverse event. A TEAE was defined as an AE that presents after the initiation of treatment or any AEs already present that worsen in either intensity or frequency following treatment. Each subject was counted once for each applicable category.

Toxicity grade: Grade 1 = Mild; Grade 2 = Moderate; Grade 3 = Severe; Grade 4 = Potential life-threatening. SAE = Serious adverse event; AE = Adverse event; AESI = Adverse Event of Special Interest.

Ref: STN 125761/0/27, ISS Addendum 3, Table 2, page 15 of 57, Source: Table 14.3.1.1b; STN 125761/0/38, Table 11.1.2.1a, pages 1-2.

While the number and frequency of TEAEs reported in AV7909 vaccinated subjects in the Tier 1 and 2 analyses and in Study EBS.AVA.212 were generally similar (frequency ranged from 38.6% to 41.6%), the number and frequency of TEAEs were generally higher for all BioThrax vaccinated groups (frequency ranged from 47.1% to 88.9%). The number and frequency of TEAEs in both AV7909 and BioThrax vaccinated groups 'related to' vaccination was generally numerically lower across all groups, consistent with safety findings seen during review of each respective clinical study. Grade 3 or higher TEAEs were infrequent, with ≥Grade 3 TEAEs related to AV7909 vaccination even lower (approximately 1.3% frequency). Grade 4 TEAEs were exceedingly rare in both AV7909 and BioThrax vaccinated subjects (n=21/3151 or 0.7% and n=3/533 or 0.6% for AV7909 and BioThrax, respectively; STN 125761/0/38, ISS Addendum 3, Source: Table 14.3.1.4.1b).

Similarly, TEAEs leading to AV7909 vaccine discontinuation were infrequent (approximately 2.2% to 2.3% frequency), and even lower for TEAEs in AV7909 vaccinated subjects that led to study withdrawal (approximately 0.03% to 0.06% frequency). Two subjects (<0.1%), with one TEAE each, were withdrawn from the study due to TEAEs. In both cases, the TEAEs were deemed unrelated to AV7909. These two events were reported in the Infections and Infestations SOC with PT cellulitis (EBS.AVA.201), and in the Reproductive System and Breast Disorders SOC with PT menorrhagia (EBS.AVA.212; STN 125761/0, ISS Addendum, Source: Table

11.1.8), both previously discussed in Sections 9.2 and 6.1.12.7, respectively, in the clinical review.

Reviewer comment: Overall findings for TEAEs in AV7909 vaccinated subjects in the ISS indicate that AV7909 vaccination appeared to be well-tolerated, with few subjects discontinuing vaccination or withdrawing from the study due to reported TEAEs. Most TEAEs in AV7909 vaccinated subjects appeared to be mild-moderate in severity, with few TEAEs reported as ≥Grade 3 in severity. TEAEs were more frequent in BioThrax vaccinated subjects, with a somewhat greater proportion of ≥Grade 3 TEAEs reported in the BioThrax group; most likely reflecting the higher reactogenicity observed with SC rather than IM administration of AVA.

The exclusion of site US1027 (Study EBS.AVA.212) from the Tier 1 safety summaries did not result in any impact to the overall safety profile of AV7909. With the removal of the site's data, the proportion of subjects with TEAEs increased slightly (due to the smaller population denominator) by <1% in any of the categories examined.

A summary of the most common TEAEs (≥1% frequency) by MedDRA PT (Safety Population; Excluding site US1027) is provided in Table 37 below.

Table 37. ISS: Summary of the Most Frequent Treatment-Emergent Adverse Events (TEAEs ≥ 1%) in the Pooled To-be-marketed AV7909 Group (Tier 1) by Preferred Term (PT) (Safety Population¹)

Preferred Term	Pooled AV7909 (Tier 1) N (%) N=3276 n (%)	Pooled AV7909 (Tier 2) N (%) N=3552 n (%)	EBS.AVA.212 AV7909 Groups 1-3 N (%) N=3151 n (%)	EBS.AVA.212 BioThrax N (%) N=533 n (%)	EBS.AVA.208 BioThrax N (%) N=23 n (%)	EBS.AVA.201 BioThrax N (%) N=18 n (%)
Injection site pain	152 (4.6)	164 (4.6)	144 (4.6)	49 (9.2)	0 (0)	0 (0)
Vaccination complication	123 (3.8)	131 (3.7)	115 (3.6)	25 (4.7)	0 (0)	0 (0)
Upper respiratory tract infection	106 (3.2)	129 (3.6)	92 (2.9)	12 (2.3)	2 (8.7)	5 (27.8)
Musculoskeletal procedural complication	95 (2.9)	103 (2.9)	91 (2.9)	19 (3.6)	0 (0)	0 (0)
Procedural headache ¹	89 (2.7)	95 (2.7)	87 (2.8)	26 (4.9)	0 (0)	0 (0)
Injection site induration	75 (2.3)	84 (2.4)	70 (2.2)	122 (22.9)	0 (0)	0 (0)
Urinary tract infection	52 (1.6)	57 (1.6)	52 (1.7)	4 (0.8)	0 (0)	1 (5.6)
Headache	52 (1.6)	85 (2.4)	45 (1.4)	7 (1.3)	0 (0)	6 (33.3)
Injection site movement impairment	50 (1.5)	55 (1.5)	46 (1.5)	9 (1.7)	0 (0)	0 (0)
Injection site bruising	44 (1.3)	46 (1.3)	43 (1.4)	27 (5.1)	0 (0)	0 (0)
Back pain	39 (1.2)	43 (1.2)	37 (1.2)	6 (1.1)	0 (0)	1 (5.6)
Nasopharyngitis	38 (1.2)	44 (1.2)	30 (1.0)	6 (1.1)	1 (4.3)	0 (0)
Injection site pruritus	35 (1.1)	40 (1.1)	32 (1.0)	42 (7.9)	1 (4.3)	0 (0)
Nausea	34 (1.0)	52 (1.5)	30 (1.0)	2 (0.4)	0 (0)	2 (11.1)
Injection site erythema	30 (0.9)	34 (1.0)	29 (0.9)	17 (3.2)	0 (0)	0 (0)
Fatigue	18 (0.5)	48 (1.4)	12 (0.4)	1 (0.2)	0 (0)	8 (44.4)

N = Number of subjects in the safety population; n = Number of subjects with TEAEs; % = n/N*100.

AV7909 Groups: In EBS.AVA.201, Group 2 = AVA 0.5mL + CpG 7909 0.5mg; Group 3 = AVA 0.5 mL + CpG 7909 0.25 mg.

Group 4 = AVA 0.25mL + CpG 7909 0.5mg; Group 5 = AVA 0.25mL + CpG 7909 0.25mg. In EBS.AVA.208, Group 1 = AV7909/AV7909/Placebo; Group 2 = AV7909/Placebo/AV7909; Group 3= Three doses AV7909; Group 4 = Three half-doses AV7909. In EBS.AVA.210, Group 1 = AV7909 + ciprofloxacin; Group 2= AV7909+doxycycline; Group 3 = AV7909 only. In EBS.AVA.212, Group 1 = AV7909 Lot 1; Group 2 = AV7909 Lot 2; Group 3 = AV7909 Lot 3.

BioThrax Groups: EBS.AVA.201, Group 1; EBS.AVA.208, Group 5; EBS.AVA.212, Group 4.

¹Safety Population excluded site US1027 from Study EBS.AVA.212.

²Procedural headache defined as post-vaccination headache per STN 125761/0/42.

Data are sorted by descending order of the percentage in the pooled AV7909 with to-be-marketed regimen/formulation group.

Adverse events are coded according to MedDRA version 22.0.

Ref: STN 125761/0/27, ISS Addendum, Table 11.1.2.4a., page 1, STN 125761/0/38, Table 11.1.2.2a, page 1.

The most frequently reported TEAEs in AV7909 subjects were injection site reactions and comprised the following ($\geq 2\%$ frequency): injection site pain, vaccine complication, upper respiratory infection, musculoskeletal complication, procedural headache, and injections site induration. The majority of TEAEs were related to injection site reactions or systemic reactogenicity after vaccination. Upper respiratory tract infection was deemed unrelated to AV7909. The majority of TEAEs reported were Grade 1 or 2 in severity. A similar pattern of TEAEs was also reported in BioThrax vaccinated subjects, although certain injection site symptoms (e.g., induration, bruising, procedural headache, and injection site pruritus) were higher in the BioThrax group.

When assessed by relatedness to treatment, the most frequent TEAEs ($\geq 1\%$ frequency) for Tier 1 by MedDRA PT that were determined to be 'related' to treatment (Safety Population) were (in order of frequency): injection site pain (4.6%), vaccination complication (3.8%), musculoskeletal procedural complication (2.9%), procedural headache (2.7%), injection site induration (2.3%), injection site movement impairment (1.5%), injection site bruising (1.3%), and injection site pruritus (1.1%) (STN 125761/0/27, ISS Addendum, compiled from Table 11.1.4.1a, pages 1-15).

TEAEs deemed 'related to' AV7909 vaccination all comprised post-vaccination reactogenicity events. These were generally Grade 1-2 in severity. The most frequently reported related TEAEs were injection site pain (2.8%), vaccination complication (2.6%), procedural headache (2.2%), musculoskeletal procedural complication (1.8%), and injection site movement impairment (1.2%). No significant difference in TEAE frequencies related to AV7909 vaccination were seen between the Tier 1 and Tier 2 groups.

When analyzed by demographic subgroups, Tier 1 subjects in the 18-30 years of age subgroup reported the highest proportion of TEAEs (40.4%), followed by 31-50 years of age (39.5%), and 51-65 years of age (38.4%); (STN 125761/0/38, ISS Addendum 3, Source: Table 11.1.2.3.1a). Most subjects across all age groups reported TEAEs that were mild to moderate severity (Grade 1 and 2); \geq Grade 3 TEAEs were slightly more frequent in older subjects (5.8% in the 18-30-year-old subgroup, 6.5% in the 31-50-year-old subgroup, vs. 7.4% in the 51-65-year-old subgroup) (STN 125761/0/38, Table 11.1.3.2.1a, pages 1-114).

In addition, a slightly higher proportion of TEAEs were reported by female (43.5%) than male subjects (34.0%) in Tier 1 (STN 125761/0/38, Table 11.1.2.3.2a, pages 1-80). In addition, a slightly higher proportion of TEAEs related to AV7909 were reported by female subjects (13.6%) than male subjects (10.2%). When assessed by race, the highest proportion of Tier 1 subjects that reported TEAEs were in the 'Other' race group (45.7%), followed by White (39.7%), and Black or African American (36.7%) (STN 125761/0/38, Table 11.1.2.3.3a, pages 1-114), but similar proportions of TEAEs 'related to' AV7909 were reported in each racial subgroup. The majority of TEAEs reported across all racial subgroups were mild-moderate in severity.

Reviewer comment: TEAEs related to AV7909 vaccination primarily reflected local reactogenicity events. The frequency of TEAEs related to vaccination was generally higher in BioThrax vaccinated subjects, than in AV7909 vaccinated subjects, particularly those related to injection site reactions.

Evaluation of TEAE frequency by demographic subgroups indicated a slightly higher frequency of TEAEs in younger subjects (31-50 years of age) and in female subjects. Older aged subjects reported a slightly higher frequency of more severe TEAEs. Other than these observations, no clear pattern or trend was seen in TEAE frequency, especially those deemed 'related to' AV7909 vaccination, across demographic subgroups.

8.4.5 Clinical Test Results

Clinical laboratory evaluations were not included in the integrated safety analysis (ISS) as no clinically meaningful vaccine effects were observed in laboratory parameters following administration of AV7909. Laboratory evaluations including hematology, serum chemistry, and urinalysis were performed in the four clinical studies. Findings with respect to laboratory evaluations were reported in the individual study reports available in Module 5.3.5.1 of the BLA submission and summarized by the clinical reviewer under the appropriate section of the clinical review for each clinical study evaluated in this BLA.

Reviewer comment: There was no observed clinically significant AV7909 vaccine effect on laboratory evaluations of subjects following vaccination.

8.4.6 Systemic Adverse Events

Systemic reactogenicity results were not pooled across studies, since signs and symptoms, and toxicity grading scales used for severity rating of solicited events post-vaccination were not identical across the four AV7909 clinical studies (see prior discussion under Section 8.3 'Caveats Introduced by Pooling of Data Across Studies/Clinical Trials').

Reviewer comment: Additional reasons not to pool reactogenicity results across studies were the different timing and difference in subject populations of the respective studies, which might influence reactogenicity findings.

8.4.7 Local Reactogenicity

Similar to systemic reactogenicity events (see Section 8.4.6, above), local reactogenicity results were not pooled across studies, since e-diary and in-clinic injection site signs and symptoms and toxicity grading scales (used for solicited AE severity rating) were not identical across the four AV7909 clinical studies (e.g., induration was not recorded in Study 208) (STN 125761/0, ISS, Section 3.6.2 Local Reactogenicity, pages 35-36 of 107).

Reviewer comment: Given that the majority of solicited AE data (e.g., local reactogenicity) was provided by Study EBS.AVA.212 due to the large number of subjects enrolled, the clinical reviewer considers the reactogenicity findings from EBS.AVA.212 as best representing AV7909's local reactogenicity findings; specifically, as those that should be included in the USPI for AV7909.

8.4.8 Adverse Events of Special Interest

Fifteen confirmed AESIs were reported in 15 subjects (0.4%) in Group 2. All AESIs reported during the development of AV7909 were in subjects enrolled in Study EBS.AVA.212 and are summarized in Table 16 in Section 6.1.12.5 of the clinical review. Of the 15 AESIs reported in subjects vaccinated with AV7909, three were adjudicated as 'possibly related' to vaccination (ulcerative colitis in Subject US(b) (6) , diffuse alopecia in Subject US(b) (6) , and chronic spontaneous urticaria in Subject US(b) (6)).

In subjects who received BioThrax, one confirmed AESI of lichen planus was adjudicated as 'possibly related' to treatment (Subject US(b) (6)). The rate of AESIs between AV7909 and BioThrax were comparable (0.5% for AV7909; 0.4% for BioThrax).

8.5 Additional Safety Evaluations

8.5.1 Dose Dependency for Adverse Events

Solicited AEs (local and systemic reactogenicity) were assessed after each vaccination dose in each of the four clinical studies conducted. A review of post-vaccination solicited AE data showed no clear pattern or trend in solicited AE frequency or severity. Based on the solicited AE data across studies, it is not possible to conclude that subsequent vaccinations with AV7909 will result in more severe injection site or systemic reactions. Similarly, an evaluation of TEAEs, SAEs, and AESIs failed to establish a clear pattern or trend after vaccination with AV7909.

8.5.2 Time Dependency for Adverse Events

An evaluation of unsolicited AEs (i.e., TEAEs, SAEs, and AESIs) in the four clinical studies that comprise this BLA submission did not show a pattern or trend in AEs or time to onset (i.e., time dependency) of AEs.

8.5.5 Product-Product Interactions

AV7909 was evaluated in conjunction with ciprofloxacin and doxycycline (Study EBS.AVA.210), which are recommended first line antibacterial drugs as part of treatment and prophylaxis following potential exposure to *B. anthracis*. Safety results from this study are summarized in Section 2.2.3 of this clinical review; detailed safety information can be found in Section 12 of the CSR for Study EBS.AVA.210 CSR. There were no apparent safety concerns observed for IM administration of AV7909 vaccine either as monotherapy or in combination with ciprofloxacin or doxycycline antibacterial as summarized in Section 2.2.3.

8.6 Safety Conclusions

The ISS did not identify any new safety concerns pertaining to AV7909 administration. The safety findings in the ISS were primarily driven by the safety results from Study EBS.AVA.212. Subject disposition and demographics mostly reflected those of Study EBS.AVA.212. Additionally, the number and frequency of deaths, SAEs and AESIs in the ISS were almost identical to those reported in Study EBS.AVA.212. The majority of TEAEs reported were related to injection site reactions and were mild-moderate in severity (Grade 1 or 2).

The ISS did not provide any new information regarding the safety profile of AV7909; and confirmed Study 212's safety findings. AV7909 was found to be generally well-tolerated and without any safety signals identified.

9. ADDITIONAL CLINICAL ISSUES

9.1 Special Populations

Not applicable.

9.1.1 Human Reproduction and Pregnancy Data

No clinical studies included in this BLA pre-specified a formal evaluation of AV7909 in pregnant women. WOCBP were required to use appropriate contraception prior to, during, and post-vaccination and underwent pregnancy testing prior to each vaccination to confirm negative pregnancy status. Subjects who inadvertently became pregnant on study received no further vaccination (if applicable) and were followed to term, to collect maternal fetal outcome data.

Pregnancy Outcomes in Women of Child-bearing Potential (WOCBP)

A total of 38 pregnancies were reported in 36 female subjects (two twin gestations) who received either AV7909 (n=33) or BioThrax (n=2) in studies EBS.AVA.208, -210, and – 212, or saline placebo (n=1) in study EBS.AVA.201. The majority of female subjects (including WOCBP) in the ISS were enrolled in Study EBS.AVA.212; with the majority of pregnancies in the ISS reported from EBS.AVA.212.

For the AV7909-vaccinated female subjects who became pregnant (n=33) across all clinical studies, 11 subjects were exposed to AV7909 during pregnancy (all first in the trimester) and 22 subjects with 24 pregnancies (two twin pregnancies) were exposed to AV7909 prior to conception (PTC)/pre-pregnancy—with one subject exposed to AV7909 within 30 days of conception. Of these 35 pregnancies in AV7909-vaccinated female subjects, adverse pregnancy outcomes included 7 spontaneous abortions/miscarriage (20% incidence), 23 live births (65.7% incidence), and two live births with a major birth defect (5.7% incidence). These data are summarized in Table 38 below.

Table 38. Number and Frequency of Known Pregnancy and Fetal Outcomes in Female Subjects with Exposure to AV7909 in all Clinical Studies submitted to BLA STN 125761/0 (Studies EBS.AVA.201, 208, 210, and 212) by Timing of AV7909 Exposure

Outcome	PTC	1 st Trimester	2 nd Trimester	3 rd Trimester	Unknown	Total
Spontaneous abortion < 20 weeks	7 (20%)	0	0	0	0	7 (20%)
Elective abortion	2 (5.7%)	2 (5.7%)	0	0	0	4 (11.4%)
Elective abortion with known Cas	0	0	0	0	0	0
Ectopic	0	0	0	0	0	0
Fetal demise > 20 weeks	0	1 (2.9%)	0	0	0	0
Live Birth	15 (42.9%) ^d	8 (22.9%) ^a	0	0	0	23 (65.7%)
LB with CA	1 (2.9%)	2 (5.7%)	0	0	0	3 (8.6%)
LB with Major CA	1 (2.9%) ^b	1 (2.9%) ^b	0	0	0	2 (5.7%)
LB with Minor CA	0	1 (2.9%) ^c	0	0	0	1 (2.9%) ^c

PTC: Prior to conception; LB: Live births; CA: Congenital anomaly.

Note: Percentages (%) are based on the total number of pregnancies (35 pregnancies in 33 participants [two sets of twins])

^aIncludes 1 live-born neonate that died after 8 days of birth (infection from membranes)

^bIncludes 1 newborn with hydrocephalus, pulmonary, and bilateral renal aplasia

^cIncludes 1 newborn with a labial tie

^dIncludes 1 newborn that died within 24 hours after birth (newborn with hydrocephalus, pulmonary, and bilateral renal aplasia)

^eIncludes 1 newborn with biliary cyst

Ref: STN 125761/0/51, Table 2, pages 10-11 of 13.

A summary of all pregnancy outcomes in the ISS for all treatments administered (AV7909, BioThrax and saline placebo) by subject (including site US1027 for EBS.AVA.212) is provided in Table 39 below.

Table 39. ISS: Summary of all Pregnancy Outcomes Reported Female Subjects Across all Clinical Studies (Safety Population¹)

Study	Treatment ² Group/Lot ³	Unique Subject ID Number	Age (years)/Race	Interval from Last Dose to Positive Pregnancy Test ⁴	Interval from Last Dose to Adverse Outcome (where applicable) ⁴	Timing of Vaccine/Placebo Dose in Relation to Pregnancy	Pregnancy Outcome MedDRA Preferred Term Provided for AEs (MedDRA v22.0)	Causality ⁵
EBS.AVA.201	Placebo (saline)	(b) (6)	28 years/ Black	Approx. 1 month, 15 days	NA	Prior to Pregnancy	Birth of full term, healthy infant	NA
EBS.AVA.208	AV7909	(b) (6)	34 years/White	Approx. 6 months	NA	Prior to Pregnancy	Birth of full term, healthy infant	NA
--	AV7909	(b) (6)	25 years/White	5 months 29 days	NA	Prior to Pregnancy	Birth of full term, healthy infant	NA
--	AV7909	(b) (6)	24 years/White	3 months 8 days	10 months, 5 days	Prior to Pregnancy	Birth of premature, healthy infant (neonatal atelectasis)	Not Related
--	AV7909	(b) (6)	26 years/White	Approx. 7 months	NA	Prior to Pregnancy	Birth of full term, healthy infant	NA
EBS.AVA.210	AV7909	U (b) (6)	29 years/White	29 days	NA	First Trimester (doses 1 and 2)	Birth of full term, healthy infant	NA
EBS.AVA.212	AV7909/ Lot 3	U (b) (6)	31 years/Black	1 year 23 days ³	1 year 2 months 29 days	Prior to Pregnancy	Abortion spontaneous	Not Related
--	AV7909/Lot 1	U (b) (6)	38 years/White	Approx. 6 months 2 weeks	NA	Prior to Pregnancy	Birth of full term, healthy infant	NA
--	AV7909/Lot 2	U (b) (6)	26 years/Black	27 days	NA	Prior to Pregnancy	Birth of full term, healthy infant	NA
--	AV7909/Lot 2	U (b) (6)	34 years/Black	6 months 15 days	7 months 5 days	Prior to Pregnancy	Abortion spontaneous	Not Related
--	AV7909/Lot 2	U (b) (6)	29 years/White	1 month 9 days	NA	Prior to Pregnancy	Birth of full term, healthy infant	NA
--	AV7909/Lot 1	U (b) (6)	22 years/White	8 months 24 days	10 months 3 days	Prior to Pregnancy	Abortion spontaneous	Not Related
--	AV7909/Lot 1	U (b) (6)	33 years/Black	5 days	27 days	First Trimester (doses 1-3)	Abortion induced ⁶	Not Related
--	AV7909/Lot 2	U (b) (6)	28 years/White	3 days	6 months 17 days	First Trimester (dose 3)	Twin pregnancy: One infant: Fetal death	Not related

Study	Treatment ² Group/Lot ³	Unique Subject ID Number	Age (years)/Race	Interval from Last Dose to Positive Pregnancy Test ⁴	Interval from Last Dose to Adverse Outcome (where applicable) ⁴	Timing of Vaccine/Placebo Dose in Relation to Pregnancy	Pregnancy Outcome MedDRA Preferred Term Provided for AEs (MedDRA v22.0)	Causality ⁵
--	AV7909/Lot 2	U (b) (6)	28 years/White	3 days	NA	First Trimester (dose 3)	Twin Pregnancy: Second Infant: Birth of full term, healthy infant	NA
--	AV7909/Lot 3	U (b) (6)	25 years/Black	2 months 1 day	NA	Prior to Pregnancy	Birth of full term, healthy infant	NA
--	AV7909/Lot 2	U (b) (6)	29 years/White	2 days	23 days	First Trimester (doses 2 and 3)	Abortion induced ⁶	Not Related
--	AV7909/Lot 3	U (b) (6)	35 years/Black	15 days	NA	First Trimester (dose 2)	Birth of full term, healthy infant	NA
--	AV7909/Lot 1	U (b) (6)	34 years/Black	Unknown ⁷	6 months 14 days	Prior to Pregnancy	Abortion spontaneous	Not Related
--	AV7909/Lot 1	U (b) (6)	27 years/White	4 months 12 days	NA	Prior to Pregnancy	Birth of full term, healthy infant	NA
--	AV7909/Lot 3	U (b) (6)	28 years/Black	15 days	4 months 22 days	First Trimester (dose 1)	Premature delivery, premature rupture of membranes, neonatal infection; fetal death	Not Related
--	AV7909/Lot 2	U (b) (6)	21 years/White	8 months 11 days	NA	Prior to Pregnancy	Birth of full term, healthy infant	NA
--	AV7909/Lot 2	U (b) (6)	27 years/White	2 months 21 days ³	6 months 1 day ⁶	Prior to Pregnancy	Pulmonary hypoplasia, renal aplasia, hydrocephalus, fetal death	Not related
--	AV7909/Lot 3	U (b) (6)	22 years/White	15 days	NA	First Trimester (dose 2)	Birth of full term, healthy infant	NA
--	AV7909/Lot 2	U (b) (6)	26 years/White	2 months 5 days	2 months 7 days	Prior to Pregnancy	Abortion induced ⁶	Not related
--	AV7909/Lot 2	U (b) (6)	19 years/White	+8 months 3 days	8 months 12 days	Prior to Pregnancy	Abortion induced ⁶	Not related
--	AV7909/Lot 2	U (b) (6)	25 years/Black	14 days	NA	First Trimester (dose 1)	Birth of full term, healthy infant	NA
--	BioThrax	U (b) (6)	36 years/White	2 months 5 days	2 months 15 days	Prior to Pregnancy	Abortion spontaneous	Not related
--	BioThrax	U (b) (6)	27 years/Multiple	6 months 14 days	NA	Prior to Pregnancy	Birth of full term, healthy infant	NA
--	AV7909/Lot 3	U (b) (6)	25 years/Black	1 month 4 days	NA	Prior to Pregnancy	Birth of full term, healthy infant	NA
--	AV7909/Lot 3	U (b) (6)	32 years/White	4 months 26 days	NA	Prior to Pregnancy	Birth of full term, healthy infant	NA

Study	Treatment ² Group/Lot ³	Unique Subject ID Number	Age (years)/Race	Interval from Last Dose to Positive Pregnancy Test ⁴	Interval from Last Dose to Adverse Outcome (where applicable) ⁴	Timing of Vaccine/Placebo Dose in Relation to Pregnancy	Pregnancy Outcome MedDRA Preferred Term Provided for AEs (MedDRA v22.0)	Causality ⁵
--	AV7909/Lot 1	U (b) (6)	32 years/White	3 months 4 days	NA	Prior to Pregnancy	Birth of full term, healthy infant	NA
--	AV7909/Lot 3	U (b) (6)	29 years/White	1 month 6 days	8 months 5 days	First Trimester (dose 3)	Twin Pregnancy: One infant with biliary cyst	Possible
--	AV7909/Lot 3	U (b) (6)	29 years/White	1 month 6 days	8 months 5 days	First Trimester (dose 3)	Twin Pregnancy: Second infant with labial tie	Possible
--	AV7909/Lot 2	U (b) (6)	32 years/White	5 months 6 days	8 months 2 days	Prior to Pregnancy	Abortion spontaneous	Not related
--	AV7909/Lot 1	U (b) (6)	20 years/White	12 days	19 days	Prior to Pregnancy	Abortion spontaneous	Not related
--	AV7909/Lot 1	U (b) (6)	31 years/White	5 months 16 days	NA	Prior to Pregnancy	Birth of full term, healthy infant	NA
--	AV7909/Lot 2	U (b) (6)	29 years/White	7 months 11 days	8 months	Prior to Pregnancy	Abortion missed	Not related

¹Safety Population includes study site US1027 for EBS.AVA.212.

²Treatment Group: AV7909 (with Lot included where applicable), BioThrax, or Saline Placebo.

³AV7909 Lot provided where applicable (i.e., Study EBS.AVA.212)

⁴Interval Between Last Vaccine Dose and Event: Interval provided in months and/or days, as appropriate. NA: Not available.

⁵Causality: defined as 'Not Related, Possible, or Related', as based on the Applicant's final assessment of the relationship between study vaccination and adverse outcome.

⁶Subjects US(b) (6), US(b) (6), US(b) (6) and US(b) (6) had elective abortion (PT Abortion induced) without complications which was not an AE per EBS.AVA.212 protocol.

⁷Subject US(b) (6) discovered the pregnancy a few days prior to the miscarriage.

Ref: STN 125761/0/37, Table 1, pages 4-9; STN 125761/0/51, Table 1, pages 3-9 of 13.

One pregnancy was reported in a female subject who received saline placebo in EBS.AVA.201, with report of a full-term healthy birth.

Four pregnancies in Study EBS.AVA.208 occurred after Day 84 (after which the requirement for specified contraception use was lifted) in females administered AV7909 vaccine: two subjects in Group 1 (AV7909 IM on Days 0, 14), one subject from Group 2 (AV7909 IM on Days 0, 28), and one subject from Group 4 (AV7909 half-dose IM on Days 0, 14, 28). Three of the four pregnancies resulted in birth of healthy, full-term infants; one infant was born slightly premature with a complication of neonatal atelectasis—all deemed related to the precautionary C-section performed due to the mother's bicornuate uterus and previous C-section history.

One pregnancy was reported in an AV7909 vaccinated female subject (Group 1B: AV7909 + ciprofloxacin) in Study EBS.AVA.210, with report of a full-term healthy birth.

Pregnancy outcomes of the 32 reported pregnancies in EBS.AVA.212, as summarized in Table 39 above, comprised:

- Birth of 15 full-term healthy infants with no reported congenital anomalies.
- Birth of two full-term healthy infants with congenital anomalies (twin pregnancy: one infant born with biliary cyst; second infant born with labial tie).
- Three deaths (one fetal, two neonatal; see narratives below):
 - One stillborn birth.
 - Neonatal death due to premature rupture of membranes and multi-organ failure secondary to fungal sepsis secondary to extreme prematurity and Grade 4 intraventricular hemorrhage.
 - Neonatal death due to multiple severe congenital abnormalities (pulmonary hypoplasia, renal aplasia, hydrocephalus).
- Seven spontaneous abortions.
- Four induced abortions.
- One missed abortion.

Brief narratives of the three deaths (one fetal, two neonatal) in EBS.AVA.212 are provided as follows:

- Subject US(b) (6) : twin pregnancy outcome of a stillborn female (intrauterine fetal demise of Baby A) and birth of a full-term healthy male (Baby B) by caesarean section at 37 weeks after receipt of AV7909 Lot 2 in a 29-year-old White female (gravida 7, para 5) with a previous medical history of three cesarean sections and one prior stillbirth in a twin pregnancy. Subject reported a positive pregnancy test four days after receipt of the third dose of blinded study drug. The deceased infant's weight was within normal limits at delivery, and only one amniotic sac was ruptured artificially, with one placenta removed from the uterus implying that this was a monoamniotic monochorionic pregnancy.

Reviewer comment: *The significance of the finding of one amniotic sac implied that the twin pregnancy was a monochorionic pregnancy with one placenta and that both twins would have had the same exposure to IP.*

The investigator assessed the event of intrauterine fetal death for Baby A to be 'possibly related' to IP. The MM assessed the event to be unexpected and unrelated to IP administration. The Applicant assessed the event as probably 'not related' to exposure to blinded study drug.

Reviewer comment: *The Applicant's assessment that the stillbirth was not related to AV7909 administration was based on the following reasons: multiple births have a higher complication rate than singletons; the mother's obstetrical medical history included a previous multiple birth resulting in a stillbirth in August 2012; and fetal death in the setting of a monoamniotic monochorionic gestation suggested that both fetuses were similarly exposed to IP. Most importantly, follow-up fetal autopsy revealed changes consistent with maternal vascular malperfusion without any gross congenital anomalies indicating that malperfusion was the likely pathophysiologic event that contributed to the infant's demise.*

- Subject US(b) (6) : pregnancy outcome of PPROM at 23 weeks, 5 days due to incompetent cervix with preterm birth of a female infant by caesarean section and sequelae of multi-organ failure and death on Day 8 (post-birth) secondary to fungal sepsis due to extreme prematurity and Grade 4 intraventricular hemorrhage in a 29-year-old White female (gravida 2, para 1). Medical history included obesity, gestational diabetes, and gestational thrombocytopenia in the prior pregnancy. Subject reported a miscarriage in 2010 and a full-term vaginal delivery with no complications in 2013. Subject reported a positive pregnancy test 15 days after receipt of the first dose of AV7909 Lot 3. No further vaccinations were given.

The investigator assessed the event of premature rupture of membranes as 'not related' to IP; the investigator attributed the event to cervical dilation and infection of membranes. The MM concurred that the event of Grade 3 pre-term delivery was unexpected but not related to exposure to the single dose of vaccine. The MM also indicated that based on the information provided; a caesarean section at 23 weeks EGA, that the preterm neonate died eight days later due to a possible membrane infection, and lack of a reasonable temporal association to IP; that the event was unexpected. The Applicant assessed the event of neonatal death to be unrelated to exposure to the blinded IP, as the subject experienced obstetrical complications due to an incompetent cervix which most likely led to the event.

- Subject US(b) (6) : pregnancy outcome of neonatal death of an early term (37 weeks) male infant due to concurrent fetal anomalies (hydrocephalus, lung hypoplasia and bilateral renal agenesis) in a 27-year-old White female who received AV7909 Lot 2 and completed all vaccinations. Her prior reproductive history was not provided on the MedWatch report. Subject reported a positive pregnancy test approximately 3 months after receipt of the last dose of AV7909.

The investigator assessed the events of hydrocephalus, lung hypoplasia, and renal agenesis to be 'possibly related' to the study drug. The MM assessed the fatal event to be a complication of the concurrent fetal anomalies. The Applicant assessed the event of neonatal demise as most likely 'unrelated to' exposure to AV7909. The infant's death was deemed likely related to the underlying fetal anomalies.

Reviewer comment: *The number of fetal or neonatal deaths (3 total) reported in pregnant female subjects enrolled in EBS.AVA.212 was low, though each case was reported in a AV7909 vaccinated subject. The first two deaths described above could clearly be attributed to causes other than administration of vaccine. The last case (Subject US(b) (6)) was more complex, though still unlikely to be related to vaccination, since the last dose of AV7909 was at least 1.5 months prior to the subject's report of pregnancy (if Day 64 was used as the subject's last report for having a negative pregnancy test). There were no fetal or neonatal deaths reported in BioThrax vaccinated female subjects.*

Congenital Abnormalities

Congenital abnormalities were reported in two pregnant subjects in EBS.AVA.212, Subject US(b) (6) and Subject US(b) (6), summarized as follows:

- Subject US(b) (6): a 29-year-old White female, gave birth to twins, a male and a female infant, each of whom had a fetal anomaly.

The subject received all doses of AV7909 (Group 3, AV7909 Lot 3). The subject's last menstrual period (LMP) was approximately three weeks prior to the last dose of vaccine. Pregnancy was complicated by dizygotic dichorionic gestation, maternal history of herpes simplex virus, and an abnormal prenatal ultrasound scan for possible choledochal cyst of the gall bladder in one fetus. The subject had complications during labor due to maternal fever and twin gestation. At 38 weeks gestation, she delivered twins: a female and a male infant via cesarean section, which was necessitated by multiple gestation failure to progress in labor.

The male infant was confirmed to have a choledochal cyst of the gallbladder upon birth, necessitating subsequent choledochocystectomy with Roux-en-Y hepaticojejunostomy due to feeding difficulties. The female infant was confirmed to have a labial tie with mild notching in the upper gum, with frenulum connecting the upper lip to upper gum. Both infants were last documented by the Applicant to be growing normally, and the AEs were considered 'resolved.' Both events were determined by the investigator to be unrelated to AV7909. The Applicant assessed these events as 'possibly related.'

- Subject US(b) (6): a 27-year-old White female received all doses of AV7909 (Lot 2). She reported her pregnancy during the study Day 120 follow-up call. At 184 days after the last dose of blinded IP was administered, the subject reported that her fetus was diagnosed with hydrocephalus and possible renal agenesis. At 37 weeks of gestational age, she delivered a male infant with pulmonary hypoplasia, hydrocephalus, and renal agenesis, who died within 1.5 hours post-birth. The event was assessed by the investigator as 'possibly related' to IP. The Applicant assessed this event as 'unrelated' to AV7909.

Reviewer comment: *In summary, pregnancy outcomes indicate that most pregnancies resulted in healthy-full term births. For pregnancy-related SAEs in female subjects, the majority of reported maternal SAEs were related to miscarriage (spontaneous abortion). Of the eleven subjects (one twin pregnancy) who were exposed to the vaccine either in the first trimester (n=10) or 30 days prior to pregnancy onset (n=1), one pregnancy (9.1%) resulted in miscarriage and there were 2 infants (18.2 %) born with major birth defects. Apart from congenital malformations seen in a twin birth (Subject US(b) (6) in Study EBS.AVA.212 (AV7909 group) which was considered 'possibly related to vaccination,' all pregnancy outcomes reported were deemed unrelated to treatment.*

The Applicant states that adverse outcomes of these pregnancies were presented and discussed with the DSMB (see EBS.AVA.212 CSR, Appendix 16.1.4.3). The DSMB cited no significant concerns regarding the safety of AV7909 in relation to pregnancy given that the number of fetal anomalies (3 in 18 live births) reported in AV7909 vaccinated subjects were reasonably close to the US national standards (1 in 33 live births).³⁸ The clinical reviewer acknowledges the difficulty of assessing relatedness of abnormal fetal outcomes to prior AV7909 vaccination; however, the low number of abnormal fetal outcomes and finding of preexisting maternal conditions in most subjects who experienced fetal anomalies appears

to indicate that vaccination did not play a significant role on maternal fetal outcomes reported in this BLA submission.

9.1.2 Use During Lactation

Not applicable.

9.1.3 Pediatric Use and PREA Considerations

Due to its orphan drug designation, evaluation of AV7909 for the PEP indication in the pediatric population was waived.

9.1.4 Immunocompromised Patients

Immunocompromised subjects were excluded from all studies submitted to the BLA; thus, no data on safety and effectiveness in this population were provided.

9.1.5 Geriatric Use

Effects of AV7909 in an elderly population (≥ 66 years of age) were reported in a non-Applicant sponsored study²¹; however, data (including source data) from this published study were not submitted with the BLA for FDA review or independently verified by FDA.

9.2 Aspect(s) of the Clinical Evaluation Not Previously Covered

Studies EBS.AVA.201 and -208 were conducted to support selection of AV7909 dose and dosing regimen for the confirmatory Phase 3 trial. In addition, these studies evaluated immunogenicity up to Day 84; a longer interval than assessed in the pivotal study EBS.AVA.212 (Day 64). Therefore, immunogenicity data from these two studies may be considered in order to inform the durability of the immune response to AV7909, when given at the to-be-licensed dose and regimen for anthrax PEP. A summary of each of these studies is provided below.

Study EBS.AVA.201

Study EBS.AVA.201 was a Phase 1, randomized, double-blind, parallel-arm, placebo-controlled, dose-ranging multicenter (3 sites) study evaluating the safety, tolerability, and immunogenicity of AV7909 in healthy adults 18-50 years of age with a study aim of identifying the optimal dose combination of AVA plus CpG.

A total of 105 subjects who met all eligibility criteria were randomized to one of six study groups, as shown in Table 40 below.

Table 40. EBS.AVA.201: Study Groups

Study Group	Investigational Product	Lot Number	Planned Sample Size
1	BioThrax	FAV304	18
2	Formulation 1 (0.5 mL AVA + 0.5 mg CpG 7909)	TC2858	18
3	Formulation 2 (0.5 mL AVA + 0.25 mg CpG 7909)	TC2859	18
4	Formulation 3 (0.25 mL AVA + 0.5 mg CpG 7909)	TC2860	18
5	Formulation 4 (0.25 mL AVA + 0.25 mg CpG 7909)	TC2861	18
6	Saline Placebo	NA	18

NA: Not Applicable

Ref: STN 125761/0, EBS.AVA.201, CSR, Table 2, page 20 of 597 and Table 4, page 22 of 597; Source: EBS.AVA.201 protocol, a copy of which is provided in Appendix 16.1.1

Each subject received an injection of the same IP (each 0.5 mL, IM) on Days 0 and 14. The total duration of subject participation in the study was 374 ± 7 days after the first vaccination.

The primary objective of the study was safety evaluation. The secondary objective was to evaluate immunogenicity, as determined by peak geometric mean TNA titer and time to peak antibody titer.

Immunogenicity assessments by TNA assay were performed at Days 0 (prior to vaccination), 7, 14 (prior to vaccination), 21, 28, 35, 42, 56, 70, 84, and/or EWW.

Safety monitoring comprised an evaluation of concomitant medication use, PE (including VS), local and systemic reactogenicity (assessed for 7 days via e-diary and assessed in-clinic), unsolicited AEs (TEAEs), SAEs, AESIs, and laboratory testing, from Day 0 through Day 84. SAEs and AESIs were followed for 12 months after the last vaccination by telephone follow-up.

Immunogenicity:

Evaluation of immunogenicity was determined by peak geometric mean TNA NF_{50} and time to peak TNA NF_{50} . Immunogenicity parameters included:

- Peak TNA (NF_{50}) and TNA (NF_{50}) titers
- Time in days to peak TNA (NF_{50})
- Proportion of subjects achieving a TNA $NF_{50} \geq 0.56$ on Days 21 and 28

Statistical Analysis

Immunogenicity findings were descriptive; there was no prespecified hypothesis testing.

Immunogenicity Results

In Study EBS.AVA.201, the percentage of subjects (Immunogenicity Population) reaching the TNA NF_{50} value of 0.56 at Days 28, 35, and 42 were 94.1% to 100% for the 0.5 mL AVA plus 0.5 mg CpG 7909 group (Group 2, Formulation 1), 93.8% for the 0.5 mL AVA plus 0.25 mg CpG 7909 group (Group 3, Formulation 2), and 88.2% to 88.9% for the 0.25 mL AVA plus 0.5 mg CpG 7909 group (Group 4, Formulation 3). The percentages steadily declined after Day 42.

Assessment of TNA (NF_{50}) GMTs by study day (data not shown; EBS.AVA.201, CSR, Table 10, pages 56-57 of 597) indicated no significant numerical change in the TNA (NF_{50}) GMTs at the Day 7 time point after the first injection of IP. Geometric mean TNA (NF_{50}) levels started to increase after Day 14 with peak levels achieved at Day 28 and gradual declining thereafter from Day 28 to Day 84. TNA (NF_{50}) GMTs in the saline placebo group remained close to baseline levels throughout the study (data not shown; EBS.AVA.201, CSR, Figure 1, page 58 of 597).

The maximum increase in TNA (NF_{50}) GMT values was observed in all AV7909 arms at Day 28, and at Day 35 for the BioThrax arm (data not shown; EBS.AVA.201, CSR, Figure 1, page 58 of 597). Formulations 1 and 2 containing 0.5 mL of AVA per dose (and 0.25 or 0.5 mg CpG 7909, respectively) trended towards higher peak GMT NF_{50} values and a greater percentage of subjects with TNA NF_{50} levels ≥ 0.56 than Formulations 3 and 4, which contained 0.25 mL of AVA per dose. Based on the percentage of subjects achieving a TNA NF_{50} threshold of ≥ 0.56 and GMT TNA NF_{50} levels, Formulations 1 and 2 which contained 0.5 mL AVA (but differing doses of CpG 7909 adjuvant) were shown to be most immunogenic of the AV7909 formulations assessed in EBS.AVA.201.

Safety Results:

Injection site reactions were the most frequently reported safety findings and were generally mild to moderate in severity. Injection site reactions were included as TEAEs; the majority of TEAEs reported were related to injection site reactions. No association was observed between TEAE rate and the amount of AVA or CpG 7909 per dose.

No SAEs related to IP were reported. No AESIs were reported. One subject (Subject (b) (6) in the saline placebo arm became pregnant during the study with subsequent birth of a healthy, full-term infant (see Section 9.1.1).

Hematology results through Day 84 showed a trend towards decreased absolute lymphocyte count (ALC) on Day 1 after the first immunization in the AV7909 groups. Lymphopenia occurred in each of the AV7909 groups: 33.3% in Group 1, 22.2% in Group 4, 17.6% in Group 2, and 5.3% in Group 3. This finding was not observed in the BioThrax or placebo groups. Differences in ALC between study groups were not notable by Day 7. Shifts from baseline in hematology parameters (Table 14.3.4.6) showed no trends observed for differences among the AV7909 formulations or in association with AVA or CpG 7909 dose. No trends were observed in serum chemistry, urinalysis, PE, or VS results through Day 84. In summary, all four formulations AV7909 showed good tolerability, with no significant safety signals identified.

Conclusions:

All four AV7909 formulations were safe and immunogenic when administered IM as a two-dose series on Days 0 and 14. The CpG 7909 adjuvant dose of 0.25 mg (Group 2) appeared to be as immunogenic as the 0.5 mg dose of CpG 7909. Peak TNA NF₅₀ responses were observed to occur at Day 28 for all the AV7909 groups (Day 35 for BioThrax), with steady decline in TNA NF₅₀ GMTs after Day 28. By Day 84, TNA NF₅₀ GMTs for all of the AV7909 groups were still higher than baseline levels, whereas TNA NF₅₀ GMTs for BioThrax was close to baseline.

Formulation 2 (0.5 mL AVA + 0.25 mg CpG 7909) had the highest geometric mean TNA peak value at Day 28 and was associated with 81.3% of subjects achieving a TNA (NF₅₀) value \geq 0.56 at Day 70. Formulation 2 also trended towards less local and systemic reactogenicity when compared with Formulation 1 (0.5 mL AVA and 0.5 mg CpG 7909). Based on the safety and immunogenicity data obtained in this study, Formulation 2 was selected for further development in Phase 2. Evaluation of duration of effect of the AV7909 formulations showed sustained increase in TNA NF₅₀ GMTs and the percentage of subjects with TNA NF₅₀ levels \geq 0.56 at Day 84 (the last time point where TNA levels were measured).

Study EBS.AVA.208

Study EBS.AVA.208 was a Phase 2, randomized, double-blind, active-controlled, parallel-arm, multicenter (4-site) study evaluating the safety and immunogenicity of AV7909 for PEP of anthrax disease in 168 healthy adults, 18-50 years of age. The purpose of this study was to assess different dosing schedules of the AV7909 formulation selected as optimal from the Phase 1 study EBS.AVA.201 (AVA 0.5 mL plus 0.25 mg CpG); when compared to half-dose AV7909 and BioThrax, to select the dosing regimen for clinical development.

Subjects were randomized using a 4:3:2:4:2 ratio to one of five groups comprising three immunization schedules (two doses, 2 or 4 weeks apart; or three doses 2 weeks apart) and two dose levels (full dose of AV7909 or half dose of AV7909), per Table 41 below.

Table 41. EBS.AVA.208: Study Groups

Study Group	Subject Number Planned, Actual	Investigational Product	Intramuscular Dosing Schedule
1	(b) (6)	AV7909 ¹	Days 0 and 14 (Placebo Day 28)
2	(b) (6)	AV7909	Days 0 and 28 (Placebo Day 14)
3	(b) (6)	AV7909	Days 0, 14, and 28
4	(b) (6)	Half Dose ² AV7909	Days 0, 14, and 28
5	(b) (6)	BioThrax	Days 0, 14, 28

¹AV7909 Full Dose: 0.5 mL (0.5 mL AVA + 0.25 mg CpG 7909); Lot TC2994 (b) (4) was used for AV7909 full and half-doses given to subjects in Groups 1-4.

²AV7909 Half Dose: 0.25 mL (0.25 mL AVA + 0.125 mg CpG 7909)

IM: Intramuscular; all doses of AV7909 and BioThrax were administered IM. For BioThrax Lot FAV392A (Emergent Product Development Gaithersburg, Inc.) given to Group 5 subjects. Each injection of placebo consisted of sterile, preservative-free saline (0.9% sodium chloride) (b) (4)

Ref: STN 125761/0, EBS.AVA, 208, CSR, Table 4, page 24 of 166.

Immunogenicity was assessed using serum samples collected (for determination of TNA NF₅₀ and seroconversion rates) on Days 0 (pre-vaccination), 21, 28 (pre-vaccination), 35, 42, 49, 63, and 84.

Safety assessment comprised an evaluation of reactogenicity (solicited systemic and injection site reactions) by subject e-diary for 7 consecutive days after each vaccination and in-clinic (Days 7, 14, 21, 28, 35, and 42, and at other visits, if applicable), by PE (including VS), and clinical laboratory tests. Serum samples were collected on Days 0 (pre-immunization), 42, and 84 for potential autoantibody testing (anti-nuclear antibodies [ANA] and RF), if any subjects reported AESIs during the study. TEAEs were assessed through Day 84 of the study. SAEs and AESIs were reported through 12 months after last vaccination.

Immunogenicity Endpoints

Primary immunogenicity endpoint:

- Defined as the LB of the 95% CIs for the percentage of subjects in each study group with Day 63 NF₅₀ values ≥ 0.56 .
 - For the primary immunogenicity analysis, success was defined by demonstration that the LB of the 95% confidence limit of the percentage of subjects with TNA NF₅₀ values ≥ 0.56 was ≥ 0.40 (40%) at Day 63. The 95% CIs for the percentages were calculated using exact binomial 95% CIs without continuity correction.

Key secondary immunogenicity endpoints comprised the following:

- Percentage of subjects in Groups 1, 3, and 4 with Day 28 TNA NF₅₀ values ≥ 0.56 .
- Percentage of subjects in each study group with Day 42 TNA NF₅₀ values ≥ 0.56 .
- Percentage of subjects achieving a specified TNA NF₅₀ value at each time point and exact Binomial 95% CIs of point estimates of percentages.
- Geometric mean of the TNA NF₅₀ values at each time point (Days 21, 28, 35, 42, 49, 63, and 84) with 95% CIs around the point estimate. The 95% CIs for the geometric mean values and ratio to geometric mean values using TNA NF₅₀ (AV7909 vs. BioThrax) obtained by using anti-log values of 95% CIs for log₁₀ TNA NF₅₀.

Reviewer comment: The design of Study EBS.AVA.208, including sample size, was informed by results from Study EBS.AVA.201.

Immunogenicity Results

The proportion of subjects (PP Population) for each study group with TNA NF₅₀ values ≥0.56 at each study visit are presented in Table 42 below.

Table 42. EBS.AVA.208: Percentage¹ of Subjects with TNA NF₅₀ ≥0.56 by Study Visit (PP Population)

TNA NF ₅₀ ≥0.56	AV7909/ AV7909/ Placebo N=44	AV7909/ Placebo/ AV7909 N=34	AV7909/ AV7909/ AV7909 N=23	½ Dose AV7909/ ½ Dose AV7909/ ½ Dose AV7909 N=44	BioThrax/ BioThrax/ BioThrax N=23
Day 0	--	--	--	--	--
n (%)	37 (0)	27 (0)	18 (0)	41 (0)	21 (0)
95% CI	0.0, 9.5	0.0, 12.8	0.0, 18.5	0.0, 8.6	0.0, 16.1
Day 21	--	--	--	--	--
n (%)	36 (41.7)	27 (22.2)	18 (50.0)	41 (7.3)	21 (4.8)
95% CI	25.5, 59.2	8.6, 42.3	26.0, 74.0	1.5, 19.9	0.1, 23.8
Day 28¹	--	--	--	--	--
n (%)	37 (83.8)	27 (11.1)	18 (94.4)	18 (94.4)	21 (47.6)
95% CI	68.0, 93.8	2.4, 29.2	72.7, 99.9	72.7, 99.9	25.7, 70.2
Day 35	--	--	--	--	--
n (%)	35 (85.7)	27 (88.9)	18 (94.4)	40 (90.0)	18 (55.6)
95% CI	69.7, 95.2	70.8, 97.6	72.7, 99.9	76.3, 97.2	30.8, 78.5
Day 42	--	--	--	--	--
n (%)	37 (86.5)	26 (100.0)	18 (94.4)	41 (97.6)	20 (70.0)
95% CI	71.2, 95.5	86.8, 100.0	72.7, 99.9	87.1, 99.9	45.7, 88.1
Day 49	--	--	--	--	--
n (%)	37 (78.4)	24 (100.0)	18 (94.4)	41 (97.6)	20 (70.0)
95% CI	61.8, 90.2	85.8, 100.0	72.7, 99.9	87.1, 99.9	45.7, 88.1
Day 63	--	--	--	--	--
n (%)	37 (56.8)	27 (100.0)	18 (100.0)	41 (90.2)	21 (52.4)
95% CI	39.5, 72.9	87.2, 100.0	81.5, 100.0	76.9, 97.3	29.8, 74.3
Day 84	--	--	--	--	--
n (%)	37 (40.5)	26 (92.3)	18 (83.3)	39 (74.4)	19 (36.8)
95% CI	24.8, 57.9	74.9, 99.1	58.6, 96.4	57.9, 87.0	16.3, 61.6

CI = confidence interval. N= sample size which varies due to availability of valid immunogenicity samples, n = sample size meeting threshold criterion (TNA NF₅₀ ≥ 0.56).

Vaccinations (0.5 mL or half-dose 0.25 mL) were administered via the IM route on Days 0, 14, and 28.

¹For AV7909/AV7909/Placebo + AV7909/AV7909/AV7909 at Day 28, the percent of subjects with TNA NF₅₀ ≥ 0.56 was 87.3% (95% CI 75.5, 94.7).

Ref: STN 125761/0, EBS.AVA.208, CSR, Table 10, pages 85-86 of 166; Source: Table 14.2.1

For the primary immunogenicity analysis, the highest percentage of subjects with GMTs for TNA NF₅₀ ≥0.56 at Day 63 was observed in the AV7909, Day 0 and 28, and Day 0, 14, and 28 dosing regimens (Groups 2 and 3; both 100.0%), followed by the AV7909 half-dose regimen (Group 4, 90.2%), the AV7909 Day 0 and 14 regimen (Group 1, 56.8%), and lastly, the BioThrax 3-dose IM regimen (Group 5, 52.4%). The corresponding 95% CI LB values in descending order were 87.2% for Group 2, 81.5% for Group 3, 76.9% for Group 4, 39.5% for Group 1, and 29.8% for Group 5. The primary outcome measure of achieving a 95% CI LB of at least 40% was successfully met by all AV7909 arms, except for the AV7909 Day 0 and 14 group (which just missed the success criterion at 39.5%) and BioThrax IM group (Days 0, 14, and 28).

Reviewer comment: *The 2-dose AV7909 vaccine schedule at Day 0 and 14 (Group 1) showed a similar immune response to the 3-dose BioThrax vaccine schedule (Group 5). The kinetics of the AV7909 immune response indicate peak immune response at Day 42 by the primary immunogenicity endpoint (i.e., 4 weeks after administration of the 2nd AV7909 dose), with subsequent decline thereafter. The 2-dose AV7909 and 3-dose BioThrax vaccine schedules had similar kinetics throughout the study evaluation period with waning titers post*

Day 49. Although TNA titers wane over time, the anamnestic antibody response would be anticipated to protect against future exposure to *B. anthracis* or active infection (BLA STN 103821/5344). TNA thresholds are not expected to fully reflect the extent of protection against anthrax disease after an individual has completed the vaccine series and a sufficient interval of time has allowed B cell maturation with production of high affinity, antigen-specific neutralizing antibodies.

Select Secondary Immunogenicity Endpoint Results

The secondary immunogenicity outcome measure comparing TNA NF₅₀ GMTs for AV7909 versus BioThrax at each study visit are presented in Table 43 below.

Table 43. EBS.AVA.208: TNA NF₅₀ GMTs by Study Visit (PP Population)

Time Point	Parameter	AV7909/ AV7909/ Placebo N=44	AV7909/ Placebo/ AV7909 N=34	AV7909/ AV7909/ AV7909 N=23	½ Dose AV7909/ ½ Dose AV7909/ ½ Dose AV7909 N=44	BioThrax/ BioThrax/ BioThrax N=23
Day 0	N	37	27	18	41	21
--	GMT	0.0320	0.0320	0.0320	0.0320	0.0320
--	95% CI	0.0, 9.5	0.0, 12.8	0.0, 18.5	0.0, 8.6	0.0, 16.1
Day 21	N	36	27	18	41	21
--	GMT	0.4321	0.2661	0.6076	0.1027	0.0639
--	95% CI	0.2767, 0.6749	0.1639, 0.4321	0.2533, 1.4578	0.0687, 0.1536	0.0346, 0.1178
Day 28	N	37	27	18	18	21
--	GMT	1.8301	0.1913	2.1421	0.7112	0.3980
--	95% CI	1.3229, 2.5318	0.1288, 0.2843	1.1757, 3.9030	0.5124, 0.9871	0.2128, 0.7446
Day 35	N	35	27	18	40	18
--	GMT	1.7776	2.1595	4.0619	1.5346	0.6334
--	95% CI	1.2619, 2.5041	1.4111, 3.3050	2.5236, 6.5378	1.1711, 2.0111	0.3567, 1.1247
Day 42	N	37	26	18	41	20
--	GMT	1.2733	5.2972	4.0081	2.4030	1.1681
--	95% CI	0.9513, 1.7043	4.0929, 6.8559	2.5681, 6.2556	1.9103, 3.0227	0.7436, 1.8350
Day 49	N	37	24	18	41	20
--	GMT	0.9939	4.0152	2.9698	1.7399	0.9863
--	95% CI	0.7558, 1.3071	3.1113, 5.1818	1.9877, 4.4373	1.3944, 2.1712	0.6358, 1.5301
Day 63	N	37	27	18	41	21
--	GMT	0.6732	2.5746	2.0526	1.1933	0.6225
--	95% CI	0.5043, 0.8987	1.9425, 3.4122	1.3695, 3.0765	0.9455, 1.5061	0.4119, 0.9407
Day 84	N	37	26	18	39	19
--	GMT	0.4474	1.5389	1.3449	0.7327	0.4072
--	95% CI	0.3323, 0.6023	1.1747, 2.0160	0.8726, 2.0729	0.5751, 0.9337	0.2471, 0.6710

CI = confidence interval, N= sample size which varies due to availability of valid immunogenicity samples.

Vaccinations (0.5 mL or half-dose 0.25 mL) were administered via the IM route on Days 0, 14, and 28.

Group 1=AV7909/AV7909/Placebo; Group 2=AV7909/Placebo/AV7909; Group 3=AV7909/AV7909/AV7909

Group 4=½ dose AV7909/ ½ dose AV7909/ ½ dose AV7909; Group 5=BioThrax/BioThrax/BioThrax.

GMT = Geometric Mean Titer.

TNA NF₅₀ values below the lower limit of quantitation (LLOQ) are reported as 0.032, which is ½ the LLOQ, for the GMT and 0.064, which is the LLOQ, for seroconversion.

Ref: STN 125761/0. EBS.AVA.208, CSR, Table 14.2.1, pages 1-9; Data Source: Listing 16.2.3.1 and 16.2.6.1

With vaccinations given on Days 0, 14, and 28, GMT TNA NF₅₀ levels were observed to increase after Day 0 (first injection) in all study groups and continued to increase until peak

levels were achieved at Day 28 for Group 1, at Day 35 for Group 3, and at Day 42 for Groups 2, 4, and 5. GMT TNA NF₅₀ levels were all observed to gradually decline by Day 84. The highest GMT peak for TNA NF₅₀ occurred in Group 2 subjects, followed by Group 3, Group 4, Group 1, and Group 5.

Reviewer comment: *Kinetics of the AV7909 immune response when assessed by GMTs also indicate peak immune response at Day 42 and a similar decline in GMTs for the 2-dose regimen of AV7909 given on Week 0 and 2 and the 3-dose BioThrax regimen.*

Because the anamnestic immune response is critical at later time points post-vaccination for protection against disease due to B. anthracis exposure, and a correlate of protection against anthrax disease using TNA NF₅₀ GMTs has not been established,³ GMTs are primarily useful in showing that the immune response to AV7909 is similar to that after BioThrax vaccination—a vaccine with an established record of protection against anthrax disease that is licensed for PEP against anthrax disease.^{1,3} Because the Applicant deemed ease of administration and likelihood of follow-up for subsequent vaccination during a mass anthrax exposure event a critical factor in a PEP setting, the two-dose regimen of AV7909 given over a two-week interval was considered the optimal dosing regimen for anthrax PEP, especially since AV7909 administration would be adjunct treatment to required antimicrobial therapy and because local and systemic reactogenicity with this AV7909 dosing regimen was less frequent and severe than observed with the other AV7909 dosing regimens, particularly the three-dose AV7909 regimen.

Safety Results:

Of the 168 subjects in the Safety Population, most subjects (76.8%) experienced an AE, though most AEs were mild to moderate in severity across all study groups. The AEs with the highest incidence in all study subjects (≥7%) were upper respiratory tract infection, increased respiratory rate, decreased diastolic blood pressure, and nasopharyngitis. Injection site reactions were frequent post-vaccination, but generally mild to moderate in severity. For both e-diary and in-clinic evaluation of injection site reactions and systemic reactions, following all 3 vaccinations, there was no discernible pattern regarding incidence or severity across study arms that indicated a safety concern for any of the study groups.

There were no deaths in the study. There were 3 subjects with 4 SAEs, all considered by the investigator to be unrelated to IP. There were no AESIs of potential autoimmune etiology reported through the 12-month safety follow-up phone contact after the last scheduled vaccination.

Four pregnancies were reported after Day 84 for EBS.AVA.208, when birth control restrictions were no longer in place. Pregnancies for Study 208 are summarized in Section 9.1.1.

In conclusion, the proposed PEP dosing regimen of AV7909 given at Days 0 and 14 (along with the 3-dose AV7909 dosing regimen at Days 0, 14, and 28) showed the most rapid initial increase in GMT TNA NF₅₀ and increase in the percentage of subjects with a NF₅₀ ≥0.56 at Day 21 of the AV7909 regimens evaluated and when compared to the 3-dose IM regimen of BioThrax, an important component of protection against inhalational anthrax in the immediate post-exposure interval.

AV7909 was generally well tolerated across all the dosing regimens, with no safety signals identified in EBS.AVA.208.

10. CONCLUSIONS

The Applicant submitted clinical data from four clinical studies, EBS.AVA.201, 208, 210, and 212. Studies EBS.AVA.201 and -208 established the appropriate dose and dosing schedule of AV7909 of (b) (4) AVA plus (b) (4) mg of CpG 7909 given IM at Days 0 and 14 for the PEP indication and showed that AV7909 was most immunogenic on Day 42, i.e., four weeks post-vaccination. Subsequently, TNA NF₅₀ levels were further evaluated in a larger, pivotal Phase 3 study, EBS.AVA.212, where resultant human antibody levels were bridged to protective antibody levels derived from animal challenge studies (rabbit GUP Study 646 and NHP Study 844) to support licensure under the Animal Rule.

Two sets of primary immunogenicity endpoints were evaluated in EBS.AVA.212; one to establish lot consistency, the other to demonstrate immunogenicity of AV7909 at a clinically relevant time point (Day 64) using a non-inferiority comparison to BioThrax. The percentage of subjects with TNA thresholds (TNA NF₅₀ ≥0.56 and ≥0.29) that correlated with 70% survival in two appropriate animal species (rabbit and NHPs, respectively) were assessed by immunobridging of human-to-animal immune responses. The pre-specified criteria for the two AV7909 immunogenicity co-primary endpoints at Day 64 were met, thereby demonstrating both lot consistency across the three AV7909 lots and a protective level of immunogenicity at 7 weeks (Day 64) after IM administration of the second dose of AV7909; an immune response was comparable to that of the licensed vaccine, BioThrax.

Safety of the proposed AV7909 dosing was demonstrated, with most AEs in EBS.AVA.212 related to local and systemic reactogenicity, generally rated mild to moderate in severity by study subjects. No new safety signals were identified in this study regarding administration of AV7909.

Study EBS.AVA.210 evaluated potential interference of AV7909 administration on the PK profiles of ciprofloxacin and doxycycline and conversely, the effect of ciprofloxacin and doxycycline administration on the immune response after vaccination with the PEP schedule of AV7909. In consultation with CDER, CBER concluded that Study EBS.AVA.210 demonstrated that there was no clinically significant interference of AV7909 on the PK of ciprofloxacin and doxycycline. Administration of ciprofloxacin and doxycycline did not decrease the immunogenicity of AV7909 when dosed using the PEP schedule. Safety evaluation revealed no new safety signals, with most AEs reported being mild to moderate local and systemic reactogenicity events.

11. RISK-BENEFIT CONSIDERATIONS AND RECOMMENDATIONS

11.1 Risk-Benefit Considerations

Table 44 summarizes a qualitative risk-benefit assessment for use of AV7909 vaccine for the PEP indication against suspected or confirmed anthrax exposure based upon the individual judgment of the clinical reviewer.

Table 44. Risk-Benefit Evaluation of AV7909 (Anthrax Vaccine Adsorbed plus CpG 7909)

Decision Factor	Evidence and Uncertainties	Conclusions and Reasons
<p>Analysis of Condition</p>	<ul style="list-style-type: none"> • Anthrax is a potentially fatal disease, even when treated appropriately with recommended antimicrobial therapy. • The case-fatality rate for patients with appropriately treated cutaneous anthrax is <1%, but for inhalation or gastrointestinal tract disease, mortality often exceeds 50% and approaches 100% for meningitis in the absence of antimicrobial therapy.¹⁰ • From 1900 to October 2001, there were 18 identified cases of inhalational anthrax in the US, the latest of which was reported in 1976, with an 89% (16/18) mortality rate. Most of these exposures occurred in industrial settings, (e.g., textile mills). From October 4, 2001 to December 5, 2001, a total of 11 cases of inhalational anthrax linked to intentional dissemination of <i>B. anthracis</i> spores were identified in the US, with 5 of these cases fatal. • While the incubation period is typically ≤1 week for cutaneous or gastrointestinal tract anthrax, it may be longer for inhalational anthrax (range 1-43 days) because of spore dormancy and slow clearance from the lungs, thereby potentially delaying early treatment of disease. Once patients manifest pulmonary symptoms, progression of inhalational anthrax is often rapid and lethal. • Because anthrax disease may be biphasic in some cases, with a period of improvement between prodromal symptoms and overwhelming illness, appropriate evaluation and treatment may be delayed, resulting in a significantly lower likelihood of successful treatment and higher likelihood of serious morbidity and mortality. • <i>B. anthracis</i> is one of the most likely agents to be used as a biological weapon, because: (1) its spores are highly stable, (2) spores can infect via the respiratory route, and (3) resultant inhalational anthrax has a high mortality rate. • <i>B. anthracis</i> strains resistant to one or more antibiotics have been described in the published literature¹³⁻¹⁷; a material threat determination of antibiotic-resistant anthrax was issued by the Secretary of the Department of Homeland Security on September 22, 2006. Laboratory generation of multi-drug resistant (MDR) anthrax involves relatively straightforward methodology that does not require a high level of microbiologic knowledge. 	<ul style="list-style-type: none"> • Anthrax is a potentially fatal disease, with an especially high mortality seen with inhalational anthrax. • Because of the potential for a long incubation period and biphasic clinical response, evaluation and treatment for anthrax may be delayed, thereby decreasing the likelihood of successful treatment. • <i>B. anthracis</i> is a likely candidate for biological weaponry because of its innate chemical properties, ability to infect many individuals at a low dose, and high mortality rate due to inhalational anthrax. • The potential for MDR anthrax warrants consideration of alternative post-exposure treatment approaches against <i>B. anthracis</i> strains resistant to one or more antibiotics.¹⁵⁻¹⁷

Decision Factor	Evidence and Uncertainties	Conclusions and Reasons
<p>Unmet Medical Need</p>	<ul style="list-style-type: none"> BioThrax (Anthrax Vaccine Adsorbed) is the only vaccine approved in the US for post-exposure prophylaxis (PEP) of anthrax in adults 18-65 years of age, as a 3-dose subcutaneous (SC) series (Week 0, 2, and 4). The current mainstay of therapy for anthrax disease is a 60-day course of antimicrobial therapy, with ciprofloxacin and doxycycline representing first line therapies. A 60-day course of antimicrobial therapy is required to eradicate latent anthrax spores, which in non-human primates (NHPs) have shown to be viable for up to 100 days. For severe anthrax, anthrax-specific hyperimmune globulin 5% should be considered (Anthraxil, Cangene). Raxibacumab and obiltoxaximab (Anthim), which are human monoclonal antibodies targeting the protective antigen (PA) component of the lethal toxin of <i>B. anthracis</i>, were approved by the US FDA for prophylaxis of inhalational anthrax when alternative therapies are not available or are not appropriate. Given their restricted labeling indication, neither Anthrasil, raxibacumab, or obiltoxaximab are likely to be used as front-line PEP therapies, leaving antimicrobial therapy with or without post-exposure BioThrax vaccination, as the most likely regimen to be employed in an anthrax exposure scenario and underscoring the need for a combined approach to PEP of anthrax using vaccination and antibiotic therapy. In assessing the need for additional post-exposure approaches against anthrax, important considerations include the role of additional factors that may limit the effectiveness of antibiotics for PEP of anthrax. These include the time to initiation of treatment and duration of/adherence to the antibiotic regimen. Data from the 2001 anthrax letter attacks showed that adherence to the prescribed antibiotic regimen was low. Only 44% of 6178 respondents reported taking the prescribed antibiotics for at least 60 days.¹⁰ This finding shows the need for a combined approach to PEP of anthrax using vaccination and antibiotic therapy. 	<ul style="list-style-type: none"> AV7909 provides another therapeutic option for PEP against disease due to anthrax exposure for adults 18-65 years of age. The other available therapeutic class to routinely treat anthrax post-exposure comprises antimicrobial therapy. Anthrax hyperimmune IgG, raxibacumab, and obiltoxaximab have more restricted labeling indications and would not be used for routine anthrax PEP. Because compliance with 60-day antimicrobial regimens have been documented to be low and the first-line therapies (quinolones) are poorly tolerated due to gastrointestinal side effects, addition of second vaccine to prevent development of anthrax disease may improve morbidity and mortality in those individuals exposed to <i>B. anthracis</i>.
<p>Clinical Benefit</p>	<ul style="list-style-type: none"> AV7909, administered as a two-dose series, may ensure good patient compliance. Administration of AV7909 by the intramuscular (IM) route may be logistically easier for healthcare providers than SC administration of BioThrax according to statements made by the CDC in the context of a separate pre-EUA for BioThrax under the CDC-sponsored IND 18384. AV7909 vaccine has been shown in nonclinical and clinical studies to result in a robust immune response against the protective antigen (PA) of anthrax, with a peak immune response 28 days after completion of the 2-dose vaccine series (Day 42). With fewer injection site-related adverse events (AEs) observed with the IM route of administration for AV7909 compared to the SC route for BioThrax, it may be reasonable to anticipate that AV7909 administered IM may have fewer injection site-related AEs compared to the licensed BioThrax PEP regimen given by the SC route of administration. The availability of an additional vaccine for PEP in the US Strategic National Stockpile (SNS), after exposure to <i>B. anthracis</i> as a critical medical countermeasure, could mitigate against a potential preparedness gap and add to the US Government's armamentarium for treatment of anthrax. 	<ul style="list-style-type: none"> AV7909 may enhance preparedness and response capabilities with greater ease of use (IM administration instead of SC) and compliance (2 doses) over the currently licensed BioThrax (3 doses) in the event of an anthrax emergency. Availability of AV7909 would provide an additional vaccine for PEP against <i>B. anthracis</i> as a medical counter measure (MCM) and would likely expand the number of doses of anthrax vaccine available in the SNS, that could be available to the US public in the event of a mass anthrax event.

Decision Factor	Evidence and Uncertainties	Conclusions and Reasons
Risk	<ul style="list-style-type: none"> In adults 18 through 65 years of age, the most common injection-site adverse reactions with AV7909 administration were tenderness, pain, warmth, and arm motion limitations. The most common systemic AEs were muscle aches, headache, and fatigue. Submitted safety data did not demonstrate any safety signal that could be associated with the addition of CpG 7909 to AVA such as diseases of autoimmune etiology, nor did it show any clinically relevant differences from the safety profile of BioThrax as presented in the ISS Addendum. Though no safety signals pertaining to increased autoimmunity after AV7909 vaccination were observed in clinical studies, a theoretical potential for exacerbation of underlying autoimmune disease due to increased proinflammatory effects of CpG 7909 is a potential safety concern. 	<ul style="list-style-type: none"> The totality of the data submitted indicates that the risks of vaccination with AV7909 are similar to that of licensed vaccines. AV7909 has a favorable benefit/risk profile for PEP of anthrax disease following a potential/confirmed <i>B. anthracis</i> exposure.
Risk Management	<ul style="list-style-type: none"> The most substantial risks of vaccination with AV7909 are associated with the inflammation produced at the injection site. Pain, induration, movement impairment, and erythema are very common. However, most injection site reactions are mild in severity, and they resolve relatively quickly and without sequelae. No other safety signals were apparent in healthy adults 18-65 years of age. 	<ul style="list-style-type: none"> If AV7909 were approved for healthy adults 18-65 years of age, routine measures, such as the package insert and the current pharmacovigilance plan, would be adequate to manage the risks. AV7909 should only be administered to pregnant women if the benefits of vaccination (prevention of anthrax infection) outweigh any risks to the fetus (immunologic effects in the fetus not fully known at this time but there appears to be no increased risk over baseline risk of fetal congenital malformations).

11.2 Risk-Benefit Summary and Assessment

Data submitted to this original BLA establish a reasonable likelihood of benefit in humans when AV7909 is administered in combination with a 60-day course of appropriate antimicrobial therapy, for PEP of disease resulting from suspected or confirmed *B. anthracis* exposure in persons 18-65 years of age.

Although AV7909 administration has been associated with a high frequency of local reactogenicity events in individuals vaccinated with the PEP regimen, most local reactions were mild to moderate in severity, with a somewhat higher incidence in women. The benefit of protection against a fatal disease significantly outweighs the risks of cutaneous reactions at the injection site and other known adverse reactions from AV7909. Furthermore, addition of AV7909 to antibiotics for PEP may provide substantial benefit in preventing anthrax disease for individuals where poor compliance with antimicrobial therapy is documented and particularly at later time points when antimicrobial therapy has been completed and residual spores are likely to germinate (i.e., after Day 60).

11.3 Discussion of Regulatory Options

The pathway for licensure for this BLA is the Animal Rule (21 CFR 601 Subpart H for Biologics) in which human immunogenicity data are bridged to immunogenicity threshold data obtained from two relevant animal species that is associated with a 70% probability survival when animals are exposed to a lethal dose of anthrax. Evaluation of this vaccine under the traditional approval pathway is not feasible because it is not ethical to study this vaccine in humans by purposefully exposing them to anthrax and it is impractical to conduct field studies of the vaccine because the natural incidence of anthrax disease in humans is very low. Under the Animal Rule, as a PMR the Applicant must verify and describe AV7909's clinical benefit and assess its safety when used for PEP in a field study in humans to be conducted in the event of exposure to anthrax due to a bioterror or accidental release of anthrax spores. The Applicant included in this application a proposed field study to be conducted in the event of an anthrax event or high likelihood of an event. The proposed observational field study, EBS.AVA.213, was reviewed and deemed generally acceptable to meet its stated objectives.

11.4 Recommendations on Regulatory Actions

In the opinion of this clinical reviewer, the safety and immunogenicity data provided support full approval of AV7909 given on a Week 0 and 2 schedule via the IM route of administration for PEP of disease resulting from suspected or confirmed *B. anthracis* exposure, when given with the recommended course of antimicrobial therapy in adults 18 through 65 years of age. The bridging of human immunogenicity data to two appropriate animal species which showed effectiveness for the intended indication was demonstrated under the Animal Rule.

11.5 Labeling Review and Recommendations

The Applicant's proposed revised USPI included safety and immunogenicity data from study EBS.AVA.212 and appropriate bridging data from the two pivotal animal studies, rabbit Study 646 and NHP (cynomolgus macaque) Study 844. It was amended to reflect revised safety findings (numerical changes, primarily for reactogenicity and TEAEs) from the corrected AE and CE safety datasets and submitted under BLA STN 125761/0/27 as an annotated Word and PDF copy of the USPI which also included the proposed Patient Fact Sheet (provided as one document). Summary safety information from Study EBS.AVA.212 was added to the section for PEP under 'Clinical Trials Experience' (Section 6.1.) A summary of the non-interference effects

of coadministration of ciprofloxacin and doxycycline and AV7909, as demonstrated in the pharmacokinetic Study EBS.AVA.210, was provided in Section 7.1 of the USPI. Pregnancy outcomes for BioThrax were provided in Section 8.1 (Pregnancy) of the USPI.

Labeling negotiations requested that the Applicant:

- Remove safety data from Site US1027 for Study EBS.AVA.212 from all relevant text and tables in Section 6.1.
- Provide revised reactogenicity tables to present non-pooled reactogenicity data in Section 6.1.
- Exclude any subjects from the solicited safety analysis who were reported as 'missing' subjects (e.g., subjects who did not provide any e-diary data) in the Safety Population for studies EBS.AVA.201, -208, -210, and -212.
- Delete Sub-Section 7.1 since no clinically significant PK interactions were identified.
- Provide language recommended by the CDER PK consultant for 14.2 as follows:
 - (b) (4) 
- Provide maternal fetal outcomes data in AV7909-vaccinated subjects and a summary of maternal fetal outcomes from the BioThrax pregnancy registry in Section 8.1 of the USPI.
- Remove reference to and discussion of the published BARDA study in the elderly from Section 8.5 (Geriatric Use) of the USPI.
- Include AE information for BioThrax in Section 6.2. 'Postmarketing Experience' of the USPI.

All issues, including those listed above, were acceptably resolved after exchange of information and discussions with the Applicant.

11.6 Recommendations on Postmarketing Actions

The Applicant submitted a protocol synopsis for a postmarketing study ("A Phase 4 Retrospective Observational Study of AV7909 Anthrax Vaccine Post-Exposure Prophylaxis Following a Bacillus Anthracis Mass Exposure Event"; EBS-AVA-213), which would be performed as a PMR, should a mass anthrax exposure event occur. The Phase 4 field observational study is planned to satisfy the anticipated postmarketing requirement for a product approved under the Animal Rule in the event AV7909 is deployed for a mass exposure anthrax attack. Evaluation of clinical benefit and safety from such a study will be used to further define the benefit-risk profile of AV7909 (see Module 1.17.2 for protocol synopsis).

CBER's Dr. Jane Woo, DPV determined that a REMS was not necessary. Similar to the proposed field study for BioThrax for PEP, the Applicant plans to conduct a field study to evaluate the efficacy and safety of BioThrax for the PEP indication when administered concurrently with a licensed regimen of antimicrobials following a suspected and/or confirmed exposure to *B. anthracis*.