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Randomized, double-blind, placebo-controlled, safety and immunogenicity study of 4 formulations of Anthrax Vaccine Adsorbed plus CPG 7909 (AV7909) in healthy adult volunteers☆

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ABSTRACT

A new anthrax vaccine that could accelerate the immune response and possibly reduce the number of injections needed for protection would be desirable in a post-exposure setting.

This Phase 1 study compared the safety and immunogenicity of 2 IM doses (Days 0 and 14) of 4 formulations of AV7909 (AVA plus CPG 7909) with 2 IM doses of BioThrax® (Anthrax Vaccine Adsorbed) and 2 IM doses of saline placebo administered on Days 0 and 14.

A total of 105 healthy adults 18–50 years of age were randomized to 1 of 6 study groups: BioThrax (0.5 mL), AV7909 Formulation 1 (0.5 mL AVA + 0.5 mg CPG 7909), AV7909 Formulation 2 (0.5 mL AVA + 0.25 mg CPG 7909), AV7909 Formulation 3 (0.25 mL AVA + 0.5 mg CPG 7909), AV7909 Formulation 4 (0.25 mL AVA + 0.25 mg CPG 7909), or saline placebo (0.5 mL). All randomized subjects received at least 1 vaccination, and 100 subjects completed the trial.

After 2 doses, mean peak normalized toxin neutralizing antibody responses (TNA NF_{50}) in the AV7909 groups were higher than in the BioThrax group. Differences among the 4 AV7909 groups were not statistically significant. Subjects who received AV7909 reached peak titers on Day 28 vs. Day 35 in the BioThrax group.

The most common adverse events (AEs) in the BioThrax and AV7909 groups assessed as related to vaccination were injection site reactions. Transient lymphopenia was observed after the first dose in each AV7909 group. Frequencies of injection site and systemic reactions recorded by subjects in diaries for 7 days after each injection were highest with AV7909 Formulation 1. No AEs of special interest (autoimmune events) were observed in the study.

Further studies of doses and dosing regimens are planned to assess the immunogenicity and reactogenicity of AV7909.

Abbreviations: ACIP, Advisory Committee on Immunization Practices; AE, adverse event; ALC, absolute lymphocyte count; ANOVA, analysis of variance; AVA, Anthrax Vaccine Adsorbed; AV7909, AVA plus CPG 7909; CI, confidence interval; CPG, cytosine-phosphate-guanine oligonucleotide; CRP, C-reactive protein; EBS, Emergent BioSolutions Inc.; ELISA, enzyme-linked Immunosorbent assay; GI, gastrointestinal; GMT, geometric mean titer; HLA, human leukocyte antigen; IFN, interferon; IL, interleukin; IM, intramuscular; IP, interferon-inducible protein; NF_{50}, 50% neutralization factor; ODN, oligodeoxynucleotide; PA, protective antigen; PBMC, peripheral blood mononuclear cells; PEP, post-exposure prophylaxis; rPA, recombinant protective antigen; SAE, serious adverse event; SC, subcutaneous; SD, standard deviation; SEM, standard error of the mean; Th1, type of T helper cell; TNA, toxin neutralizing antibody; TRL, toll-like receptor.

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1. Introduction

The goal of the AV7909 (AVA + CPG 7909) product development program is to create a new anthrax vaccine that will accelerate the immune response and reduce the number of injections needed to confer protective immunity in a post-exposure setting.

The results of a previous study [1] showed that mixing 1 mg of the vaccine adjuvant CPG 7909 [2–4] with BioThrax® (Anthrax Vaccine Adsorbed) (0.5 mL) just prior to intramuscular (IM) administration on Days 0, 14 and 28 significantly increased anthrax anti-protective antigen (PA) antibody and toxin neutralizing antibody (TNA) concentrations, and significantly accelerated the development of antibody by at least 3 weeks as compared with BioThrax alone. However, there was a trend toward a greater frequency and severity of AEs in the BioThrax + CPG 7909 group compared with either BioThrax or CPG 7909 alone.

The objectives of the current study were to evaluate the safety (primary) and immunogenicity (secondary) of 2 IM doses of each of 4 formulations of AV7909 compared with 2 IM doses of BioThrax or saline placebo administered on Days 0 and 14. The amount of CPG 7909 in each AV7909 formulation (0.25 mg or 0.5 mg) and the amount of AVA in AV7909 Formulations 3 and 4 (0.25 mL) were lower than the amounts used in a previous Phase 1 study (0.5 mL BioThrax and 1.0 mg CPG 7909) [1]. This was done to identify an AV7909 formulation that elicits increased immunogenicity without increased reactogencity as compared with BioThrax when administered via the IM route as post-exposure prophylaxis (PEP).

2. Materials and methods

2.1. Investigational products

All investigational products were administered IM in the deltoid muscle using a 1 inch or 1.5 inch, 23 or 25 gauge sterile needle.

BioThrax (Emergent BioSolutions, Lansing, MI) is prepared from cell-free culture filtrates of an avirulent, nonencapsulated strain of *Bacillus anthracis*. The final product contains culture fluid proteins including the anthrax cell-binding protective antigen (PA), 1.2 mg/mL aluminum as adjuvant, and 25 mcg/mL benzethonium chloride and 100 mcg/mL formaldehyde as preservatives [5]. One BioThrax dose was 0.5 mL. The BioThrax lot number was FAV304.

AV7909 final drug product is made from AVA bulk product in combination with CPG 7909. The contents of the AV7909 formulations were as follows: Formulation 1 (0.5 mL AVA + 0.5 mg CPG 7909), Formulation 2 (0.5 mL AVA + 0.25 mg CPG 7909), Formulation 3 (0.25 mL AVA + 0.5 mg CPG 7909), and Formulation 4 (0.25 mL AVA + 0.25 mg CPG 7909). All AV7909 formulations were pre-formulated to include AVA and CPG 7909 with a final volume of 0.5 mL per dose. AV7909 vaccine lot numbers were as follows: Formulation 1 (TC 2858), Formulation 2 (TC 2859), Formulation 3 (TC 2860), and Formulation 4 (TC 2861).

CPG 7909 is a 24-mer single-stranded immunostimulatory synthetic oligodeoxynucleotide (ODN) of sequence 5′-TCGTCGTTTTGTCGTTTTGTCGTT-3′. CPG 7909 contains phosphorothioate linkages to afford resistance to degradation by endogenous nucleases [1]. Like other CpG ODNs, CPG 7909 is an agonist for toll-like receptor 9 (TLR9) and thus directly activates human B cells and plasmacytoid dendritic cells. When CpG ODNs are used as vaccine adjuvants in combination with an antigen, these direct effects, combined with cytokine-mediated indirect effects on other immune cells including CD4+ T cells, results in enhanced antigen-specific antibody responses [2,4]. The solubility of CPG 7909 in aqueous solvents is approximately 250 mg/mL [6].

The placebo was 0.5 mL of sterile, preservative-free saline for injection (0.9% sodium chloride) USP supplied in 5-mL vials.

2.2. Study design

This was a Phase 1, randomized, parallel-group, double-blind, placebo-controlled safety and immunogenicity study. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. A safety monitoring committee (SMC) consisting of 3 physicians provided safety oversight and an independent safety monitor (ISM) was available at each site to provide independent safety assessments, as needed.

A total of 105 healthy adults 18–50 years of age who provided informed consent and met the entry criteria were randomized at a ratio of 6:6:6:6:6:5 to 1 of 6 study groups: BioThrax, AV7909 Formulation 1 (0.5 mL AVA + 0.5 mg CPG 7909), AV7909 Formulation 2 (0.5 mL AVA + 0.25 mg CPG 7909), AV7909 Formulation 3 (0.25 mL AVA + 0.5 mg CPG 7909 + 0.25 mL saline), AV7909 Formulation 4 (0.25 mL AVA + 0.25 mg CPG 7909 + 0.25 mL saline), or saline placebo (0.5 mL). Prior immunization with anthrax or rPA vaccine, known exposure to *B. anthracis*, or participation in anthrax therapeutic or vaccine trials were exclusionary. Each subject was to receive 2 IM injections with the same investigational product on Days 0 and 14. Subjects were stratified by gender to ensure enrollment of at least 40% male and 40% female subjects.

2.3. Immunogenicity assessment

Blood samples for determination of vaccine immunogenicity were collected on Days 0 (pre-injection), 7, 14 (pre-injection), 21, 28, 35, 42, 56, 70, and 84. Neutralizing antibody levels in blinded serum samples were measured using a validated anthrax lethal toxin neutralization assay [7,8]. The primary assay endpoint was the 50% neutralization factor (TNA NF_{50}). TNA NF_{50} is calculated as the ratio ED_{50} of the test sample to ED_{50} of a reference serum. The reference standard, AVR801, is pooled human serum from individuals immunized with BioThrax. Values below the LLOQ (ED_{50} of 33) were replaced with one-half the LLOQ (ED_{50} of 16.5) for calculation of geometric mean titer (GMT) and statistical analysis. The analysis algorithm for the TNA assay was developed by the CDC [7]. All TNA analyses were conducted at Battelle Memorial Institute, West Jefferson, Ohio.

2.4. Safety assessment

Safety was assessed from Day 0 through Day 84 by collecting data on AEs, clinical laboratory tests (hematology, serum chemistry, and urinalysis), physical examinations, and vital signs. Safety was also evaluated using a 7-day subject diary to assess injection site and systemic reactions following each vaccination. The subject diary was reviewed in the clinic on the 2 days following the first vaccination and over the phone 2 days after the second vaccination.

Blood samples were collected 1 and 2 days after the first vaccine dose to examine biomarkers sensitive to the acute effects of CPG 7909 on innate immunity: C-reactive protein (CRP) and absolute lymphocyte count (ALC). CRP measurements were also done on samples collected pre-vaccination and on Days 1, 2, 7 and 14 to potentially assist with the assessment of systemic reactogenicity across vaccine formulations. CRP was measured using high-sensitivity latex bead-enhanced nephelometry (Roche Diagnostics, Indianapolis, IN). The reference range for the assay was 0.1–3.0 mg/L.

Data on injection site reactions (pain, itching, tenderness, swelling, or redness at the injection site and/or arm motion limitation) and systemic reactions (fatigue, muscle aching, headache, nausea/gastrointestinal upset, and fever) were collected on diary cards for 7 days after each injection. All injection site and systemic reactions were reported as AEs. Any combination of injection

Table 1
Number and percentage of subjects with treatment-related adverse events occurring through Day 84.[a]

MedDRA preferred term	BioThrax 0.5 mL (n = 18)	AV7909				Saline placebo 0.5 mL (n = 15)
		Formulation 1 AVA 0.5 mL + CPG 7909 0.5 mg n = 18	Formulation 2 AVA 0.5 mL + CPG 7909 0.25 mg n = 17	Formulation 3 AVA 0.25 mL + CPG 7909 0.5 mg n = 19	Formulation 4 AVA 0.25 mL + CPG 7909 0.25 mg n = 18	
Any related AE[b]	15 (83.3)	18 (100)	14 (82.4)	14 (73.7)	18 (100)	4 (26.7)
Injection site reaction[c,j]	15 (83.3)[e]	18 (100)	13 (76.5)	14 (73.7)[e]	16 (88.9)	2 (13.3)
Fatigue[d,h]	8 (44.4)[f]	13 (72.2)	5 (29.4)	4 (21.1)	8 (44.4)	1 (6.7)
Myalgia[d,k]	7 (38.9)[e]	10 (55.6)[e]	4 (23.5)	2 (10.5)	8 (44.4)	1 (6.7)
Headache[d,i]	6 (33.3)[e]	11 (61.1)	5 (29.4)	5 (26.3)	9 (50.0)[e]	1 (6.7)
Nausea[d]	2 (11.1)	5 (27.8)	3 (17.6)	2 (10.5)	4 (22.2)	0
WBC count decreased[l]	2 (11.1)	5 (27.8)	4 (23.5)	0	2 (11.1)	1 (6.7)
Pyrexia[d]	1 (5.6)	2 (11.1)	1 (5.9)	1 (5.3)	2 (11.1)	0
Lymphocyte count decreased[g]	0	6 (33.3)[e]	3 (17.6)	1 (5.3)	4 (22.2)	0
Chills	0	2 (11.1)	1 (5.9)	0	0	0
ALT increased	0	0	1 (5.9)	0	1 (5.6)	0

ALT = alanine aminotransferase, AE = adverse event, WBC = white blood cell.

[a] Treatment-related AEs that occurred in at least 2 study subjects from Day 0 post injection through Day 84 are shown. Some subjects had multiple AEs.

[b] Related events were those assessed by the principal investigator as definitely, probably, or possibly related to investigational product. Post hoc analyses of the active treatment groups showed no significant difference in the frequency of "any related AE" between any AV7909 group vs. the BioThrax group. The incidence of "any related AE" was significantly higher in the Formulation 1 vs. Formulation 3 group (p = 0.05) and Formulation 4 vs. Formulation 3 group (p = 0.05).

[c] Injection site reaction was recorded as a AE when any combination of pain, itching, tenderness, swelling, or redness at the injection site and/or arm motion limitation was recorded by the subject on the diary card during the 7-day period after each injection.

[d] Systemic reactions (fatigue, muscle aching, headache, nausea/GI upset, and fever) were recorded as individual AEs when recorded by the subject on the diary card during the 7-day period after each injection.

[e] For 1 of these subjects, the event was assessed as severe.

[f] For 2 of these subjects, the event was assessed as severe.

[g] Post hoc analyses of the active treatment groups showed that the incidence of lymphopenia was significantly higher in the AV7909 Formulation 1 group vs. BioThrax group (p = 0.02) and significantly higher in the Formulation 1 group vs. Formulation 3 group (p = 0.04).

[h] Post hoc analyses of the active treatment groups showed that the incidence of fatigue was significantly higher in the AV7909 Formulation 1 group vs. Formulation 2 group (p = 0.02) and Formulation 1 group vs. Formulation 3 group (p = 0.003).

[i] Post hoc analyses of the active treatment groups showed that the incidence of headache was significantly higher in the AV7909 Formulation 1 group vs. Formulation 3 group (p = 0.05).

[j] Post hoc analyses of the active treatment groups showed that the incidence of injection site reaction was significantly higher in the AV7909 Formulation 1 group vs. Formulation 2 group (p = 0.05) and Formulation 1 group vs. Formulation 3 group (p = 0.05).

[k] Post hoc analyses of the active treatment groups showed that the incidence of myalgia was significantly higher in the AV7909 Formulation 1 group vs. Formulation 3 group (p = 0.005) and Formulation 4 group vs. Formulation 3 group (p = 0.03).

[l] Post hoc analyses of the active treatment groups showed that the incidence of WBC increased was significantly higher in the AV7909 Formulation 1 group vs. Formulation 3 group (0.05).

site reactions was recorded as a single AE at the maximal severity reported. Systemic reactions were recorded as individual AEs.

Data on AEs and significant new chronic medical conditions were collected during follow-up telephone calls at 6 and 12 months.

2.5. Statistical methods and study populations

Continuous variables were summarized descriptively including number of observations (n), mean, and 95% confidence intervals (CIs) or mean change from baseline, standard deviation (SD), minimum, median, and maximum. Categorical variables were summarized using number and percentage of subjects with the characteristic of interest. Statistical analysis system (SAS) version 9.2 or higher was used to program study outputs.

The safety population included subjects who received any injection. The immunogenicity population included subjects who received both vaccinations, had immunogenicity data within the allowable window, and had no protocol violations that could affect TNA values.

The GMTs of the 4 AV7909 groups were compared using an Analysis of Variance (ANOVA) model with study group as the classification variable for Days 21, 28, 35, 42, 56, 72, and 84. The titer values were log-transformed before analysis. Two-sided p-values from the ANOVA models were reported. p-Values were only descriptively interpreted since the sample size was not based on statistical considerations.

Post hoc analyses were conducted on the safety data and are presented in the footnotes of Tables 1–3. Paired comparisons were done using Fisher's Exact Test (2-tailed). Because of the small sample sizes used in this study (17–19 subjects in the active treatment groups and 15 subjects in the saline placebo group), the results of post hoc analyses must be interpreted with caution.

Adverse events were coded to a System Organ Class and Preferred Term according to the Medical Dictionary of Regulatory Activities (MedDRA® medical dictionary), Version 14.0 (Maintenance and Support Services Organization).

3. Results

3.1. Subject disposition and demographics

A total of 237 subjects were screened, and 105 met the entry criteria and were enrolled in the study (Fig. 1). All randomized subjects received at least 1 injection of investigational product and were included in the safety population. One hundred subjects completed the Day 84 visit. Three subjects in the BioThrax group, 1 in the AV7909 Formulation 2 group, and 1 in the AV7909 Formulation 3 group did not complete the study. Of the 5 subjects who did not complete the study, 4 received the first injection only and 1 (BioThrax group) received both injections and was subsequently lost to follow-up. One subject in the BioThrax group withdrew from the study prior to the second injection because of an AE (mild upper respiratory tract infection and moderate fever, both unrelated to vaccination).

Overall, 48.6% of subjects were male, the mean age was 32.0 years, and the race was predominantly white (82.9%) followed by African American (14.3%). Baseline characteristics were evenly balanced across the 6 study groups with the exception of a higher

Table 2
Number and percentage of subjects with injection site reactions recorded on diary cards after the first and second injections.

MedDRA preferred term	BioThrax 0.5 ML (n = 18)	AV7909				Saline placebo 0.5 mL (n = 15)
		Formulation 1 AVA 0.5 mL + CPG 7909 0.5 mg n = 18	Formulation 2 AVA 0.5 mL + CPG 7909 0.25 mg n = 17	Formulation 3 AVA 0.25 mL + CPG 7909 0.5 mg n = 19	Formulation 4 AVA 0.25 mL + CPG 7909 0.25 mg n = 18	
First injection						
Any injection site reaction[a]	11 (61.1)[b]	17 (94.4)[c]	13 (76.5)	12 (63.2)	16 (88.9)	1 (6.7)
Redness	0	2 (11.1)	1 (5.9)	2 (10.5)	1 (5.6)	0
Swelling	1 (5.6)[b]	4 (22.2)[c]	1 (5.9)	1 (5.3)	2 (11.1)	0
Tenderness	10 (55.6)	16 (88.9)	11 (64.7)	11 (57.9)	13 (72.2)	1 (6.7)
Injection site pain[d]	5 (27.8)	14 (77.8)	12 (70.6)	8 (42.1)	11 (61.1)	0
Injection site itching	0	2 (11.1)	2 (11.8)	0	0	0
Arm motion limitation	8 (44.4)	13 (72.2)	8 (47.1)	9 (47.4)	10 (55.6)	0
Second injection						
Any injection site reaction	11 (61.1)[b]	16 (88.9)[c]	12 (70.6)	11 (57.9)[c]	13 (72.2)	0
Redness	1 (5.6)	3 (16.7)	2 (11.8)	3 (15.8)[b]	0	0
Swelling	2 (11.1)	4 (22.2)[b]	2 (11.8)	2 (10.5)	1 (5.6)	0
Tenderness	11 (61.1)[b]	13 (72.2)	11 (64.7)	10 (52.6)	12 (66.7)	0
Injection site pain[e]	6 (33.3)[b]	13 (72.2)[b]	10 (58.8)	8 (42.1)	6 (33.3)	0
Injection site itching	2 (11.1)	3 (16.7)[b]	3 (17.6)	2 (10.5)	0	0
Arm motion limitation	8 (44.4)[b]	11 (61.1)[b]	7 (41.2)	8 (42.1)[b]	8 (44.4)	0

[a] The results of post hoc analyses of the active treatment groups showed that after the first injection, the frequency of "any injection site reaction" was significantly higher in the AV7909 Formulation 1 group vs. BioThrax group (p = 0.04) and significantly higher in the Formulation 1 group vs. Formulation 3 group (p = 0.04).
[b] For 1 of these subjects, the reaction was assessed by the principal investigator as severe.
[c] For 2 of these subjects, the reaction was assessed by the principal investigator as severe.
[d] The results of post hoc analyses of the active treatment groups showed that after the first injection, the frequency of injection site pain was significantly higher in the AV7909 Formulation 1 group vs. BioThrax group (p = 0.007), significantly higher in the Formulation 2 group vs. BioThrax group (p = 0.02), and significantly higher in the Formulation 1 group vs. Formulation 3 group (p = 0.04).
[e] The results of post hoc analyses of the active treatment groups showed that after the second injection, the frequency of injection site pain was significantly higher in the AV7909 Formulation 1 group vs. BioThrax group (p = 0.04) and significantly higher in the Formulation 1 group vs. Formulation 4 group (p = 0.04).

Table 3
Number and percentage of subjects with systemic reactions recorded on diary cards after the first and second injections.

MedDRA preferred term	BioThrax 0.5 mL (n = 18)	AV7909				Saline placebo 0.5 mL (n = 15)
		Formulation 1 AVA 0.5 mL + CPG 7909 0.5 mg n = 18	Formulation 2 AVA 0.5 mL + CPG 7909 0.25 mg n = 17	Formulation 3 AVA 0.25 mL + CPG 7909 0.5 mg n = 19	Formulation 4 AVA 0.25 mL + CPG 7909 0.25 mg n = 18	
First injection						
Any systemic reaction[a]	10 (55.6)	12 (66.7)	7 (41.2)	3 (15.8)	7 (38.9)[b]	5 (33.3)
Fever	0	2 (11.1)	1 (5.9)	0	0	2 (13.3)
Fatigue[c]	6 (33.3)	9 (50.0)	3 (17.6)	3 (15.8)	4 (22.2)	2 (13.3)
Muscle aching[d]	4 (22.2)	8 (44.4)	3 (17.6)	0	2 (11.1)	2 (13.3)
Headache[e]	6 (33.3)	11 (61.1)	5 (29.4)	1 (5.3)	5 (27.8)[b]	2 (13.3)
Nausea/GI upset	1 (5.6)	4 (22.2)	2 (11.8)	2 (10.5)	2 (11.1)	2 (13.3)
Second injection						
Any systemic reaction[f]	7 (38.9)[b]	14 (77.8)[b]	7 (41.2)	4 (21.1)	9 (50.0)	2 (13.3)
Fever	0	0	0	0	1 (5.6)	0
Fatigue	4 (22.2)	8 (44.4)[b]	4 (23.5)	3 (15.8)	7 (38.9)	1 (6.7)
Muscle aching[g]	5 (27.8)[b]	9 (50.0)[b]	3 (17.6)	2 (10.5)	7 (38.9)	0
Headache	5 (27.8)[b]	7 (38.9)	3 (17.6)	3 (15.8)	7 (38.9)	1 (6.7)
Nausea/GI upset	1 (5.6)	3 (16.7)	0	0	3 (16.7)	0

[a] The results of post hoc analyses of the active treatment groups showed that after the first injection, the frequency of "any systemic reaction" was significantly lower in the AV7909 Formulation 3 group vs. BioThrax group (p = 0.02) and significantly higher in the Formulation 1 vs. Formulation 3 group (p = 0.003).
[b] For 1 of these subjects, the reaction was assessed by the principal investigator as severe.
[c] The results of post hoc analyses of the active treatment groups showed that after the first injection, the frequency of fatigue was significantly higher in the AV7909 Formulation 1 group vs. Formulation 3 group (p = 0.04).
[d] The results of post hoc analyses of the active treatment groups showed that after the first injection, the frequency of muscle aching was significantly higher in the AV7909 Formulation 1 group vs. Formulation 3 group (p = 0.001) and significantly lower in the Formulation 3 group vs. BioThrax group (p = 0.05).
[e] The results of post hoc analyses of the active treatment groups showed that after the first injection, the frequency of headache was significantly higher in the AV7909 Formulation 1 group vs. Formulation 3 group (p = 0.0004) and significantly lower in the Formulation 3 group vs. BioThrax group (p = 0.04).
[f] The results of post hoc analyses of the active treatment groups showed that after the second injection, the frequency of "any systemic reaction" was significantly higher in the AV7909 Formulation 1 group vs. BioThrax group (p = 0.04), Formulation 1 group vs. Formulation 2 group (p = 0.04), and Formulation 1 group vs. Formulation 3 group (p = 0.0009).
[g] The results of post hoc analyses of the active treatment groups showed that after the second injection, the frequency of muscle aching was significantly higher in the AV7909 Formulation 1 group vs. Formulation 3 group (p = 0.01).

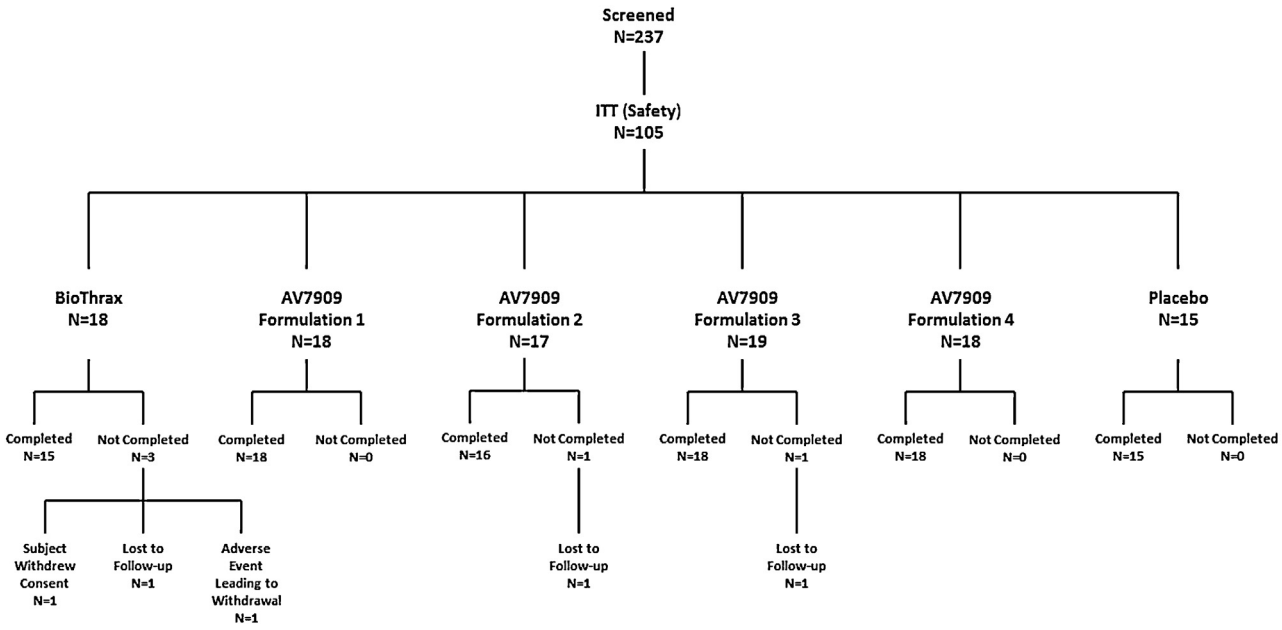

Fig. 1. Subject disposition through Day 84. A total of 237 subjects were screened within 28 days of enrollment in the study. Subjects who met the entry criteria ($n = 105$) were randomized to 1 of 6 study groups at a ratio of 6:6:6:6:6:5:BioThrax, AV7909 Formulation 1, AV7909 Formulation 2, AV7909 Formulation 3, AV7909 Formulation 4, or saline placebo. All randomized subjects received at least 1 injection of investigational product and were included in the safety population. One hundred subjects completed the study.

African American population (31.6%) in the AV7909 Formulation 3 group.

3.2. Safety

3.2.1. Adverse events

During the first 84 days of the study, 1 subject in the saline placebo group had an SAE (elevated hepatic enzymes) on Day 28. The investigator attributed this event to an acute Epstein-Barr infection because the subject had positive IgM serology for viral capsid antigen. One subject in the saline placebo group became pregnant during the study and later gave birth to a healthy, full-term infant.

All subjects who received AV7909 Formulation 1 or Formulation 4 had AEs considered to be related to vaccination, followed by 83.3% who received BioThrax, 82.4% who received AV7909 Formulation 2, 73.7% who received AV7909 Formulation 3, and 26.7% who received saline placebo (Table 1). The majority of these events were injection site reactions. Lymphopenia occurred in each AV7909 group, but not in the BioThrax or saline placebo group. Most related AEs were mild. Severe related AEs occurred in 2 subjects in the BioThrax group (1 had severe fatigue, and 1 had severe fatigue, headache, and myalgia), 2 subjects in the AV7909 Formulation 1 group (1 had severe myalgia, and 1 had severe lymphopenia), 2 subjects in the AV7909 Formulation 3 group (1 had severe injection site reaction, and 1 had severe migraine [not shown in Table 1 because this related AE occurred in a single study subject]), and 1 subject in the Formulation 4 group (severe headache) (Table 1). No AEs of special interest (autoimmune events) were observed in the study.

Follow-up data were available for 98 of 105 subjects (93.3%) at 6 months and 94 subjects (89.5%) at 12 months. A death in the AV7909 Formulation 1 group for a subject in a motor vehicle accident was reported to the investigator site during the 12-month follow-up safety telephone call. The death occurred on Day 359 and was assessed as unrelated to vaccination. An SAE of glioblastoma multiforme was reported for 1 subject in the AV7909 Formulation 2 group during the 6-month follow-up telephone call (it occurred on Day 114). The event was assessed as unrelated to vaccination. Five subjects reported AEs during the safety follow-up telephone calls at 6 and 12 months: 3 subjects (16.7%) in the AV7909 Formulation 1 group (rotator cuff syndrome, tendonitis, and facial bones fracture) and 2 subjects (11.1%) in the AV7909 Formulation 4 group (blepharospasm and multiple injuries from a horseback riding accident). Tendonitis was assessed as possibly related to vaccination, and the other events were assessed as not related. No significant new chronic medical conditions were reported during the 6- and 12-month follow-up telephone calls.

No trends for differences among study groups were observed for clinical laboratory results, vital signs, or physical examinations except for transient lymphopenia (ALC $\leq$900 cells/mm^3) in 14 subjects who received AV7909 (Formulation 1, $n = 6$; Formulation 2, $n = 3$; Formulation 3, $n = 1$; Formulation 4, $n = 4$) (Table 1). For 1 subject in the AV7909 Formulation 1 group, lymphopenia was assessed as severe, and for 1 subject in the Formulation 2 group, lymphopenia was assessed as moderate. All other cases were assessed as mild. Each case of lymphopenia started the day after the first injection and resolved within 24 h with 1 exception: 1 subject in the AV7909 Formulation 4 group had mild lymphopenia lasting 6 days. Mean percentage changes from baseline in ALC were decreases ranging from 36% to 41% for AV7909 formulations vs. 5% increase for BioThrax and 12% increase for saline placebo (data not shown).

3.2.2. Injection site and systemic reactogenicity (diary card data)

In the AV7909 groups, the percentage of subjects with injection site reactions after the first and second injections was highest with Formulation 1 (94.4% and 88.9%, respectively) and lowest with Formulation 3 (63.2% and 57.9%, respectively) (Table 2). The percentage was 61.1% for BioThrax and <10% for the saline placebo after each injection.

Across all active treatment groups, tenderness, arm motion limitation, and injection site pain were the most frequently recorded injection site reaction after each injection. After the first injection, most injection site reactions were mild in each study group (Fig. 2). One subject in the BioThrax group and 2 subjects in the AV7909 Formulation 1 group recorded severe swelling after the first injection (Table 2). After the second injection, 1 subject in the BioThrax

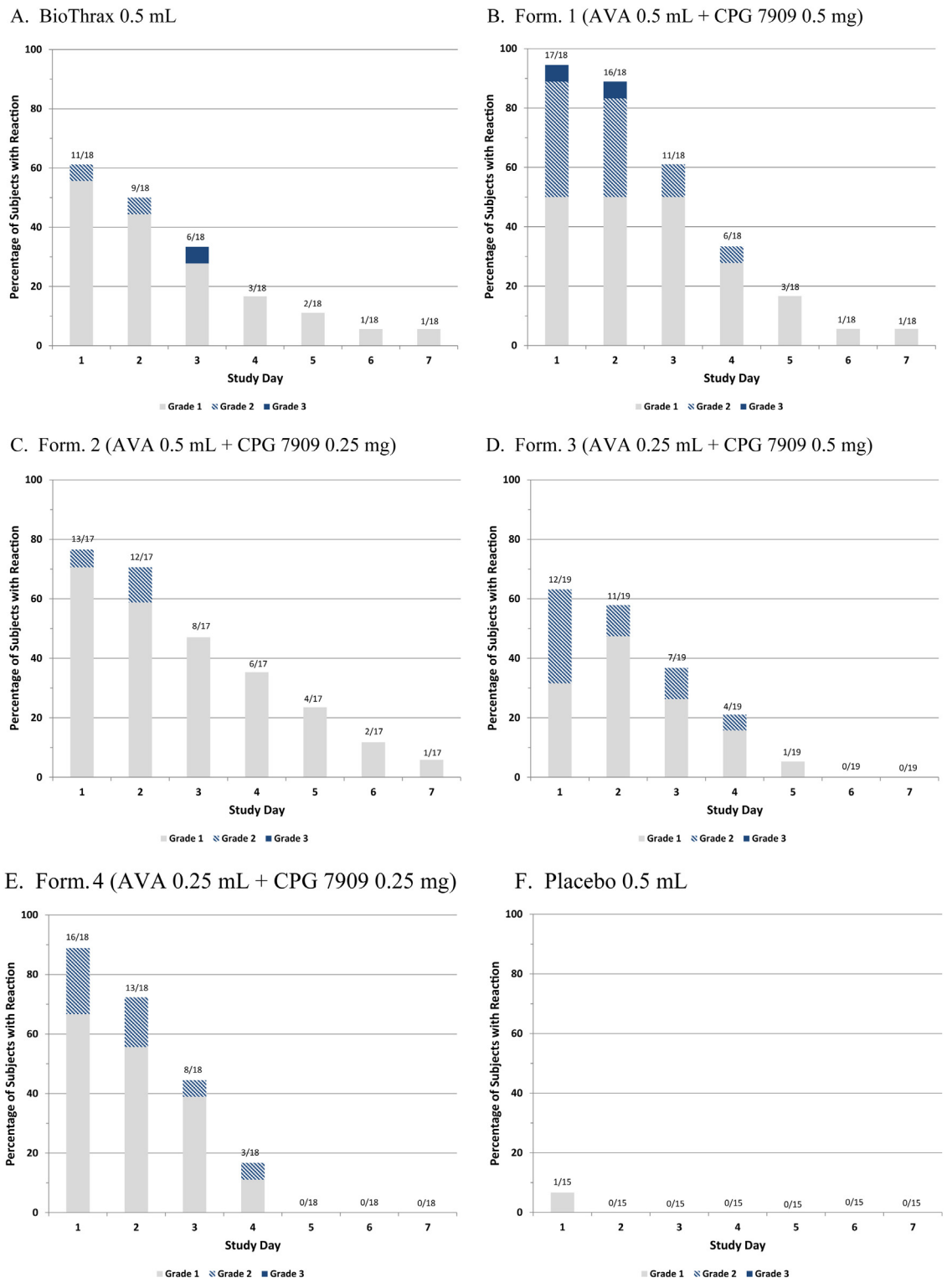

Fig. 2. Injection site reactions recorded by subjects on diary cards after the first injection. A web-enabled subject diary was to be completed by each subject for 7 days after the injection. Data were solicited on the following injection site reactions: redness, swelling, tenderness, injection site pain, injection site itching, and arm motion limitation. Each bar shows the total percentage of subjects with injection site reactions on the study day and the percentage of subjects with reactions of each severity grade (mild, moderate, severe) as applicable. Data are shown by the most severe grade if a subject had multiple events with different grades.

group, 2 subjects in the AV7909 Formulation 1 group, and 2 subjects in the AV7909 Formulation 3 group recorded severe injection site reactions.

Subjects in the AV7909 Formulation 1 group recorded the occurrence of systemic reactions on diary cards more often than subjects in the other study groups (Table 3). One subject in the AV7909 Formulation 4 group recorded a severe headache during the first 24 h after the first injection. After the second injection, 1 subject in the BioThrax group (muscle aching and headache) and 1 subject in the AV7909 Formulation 1 group (fatigue and muscle aching) recorded severe systemic reactions.

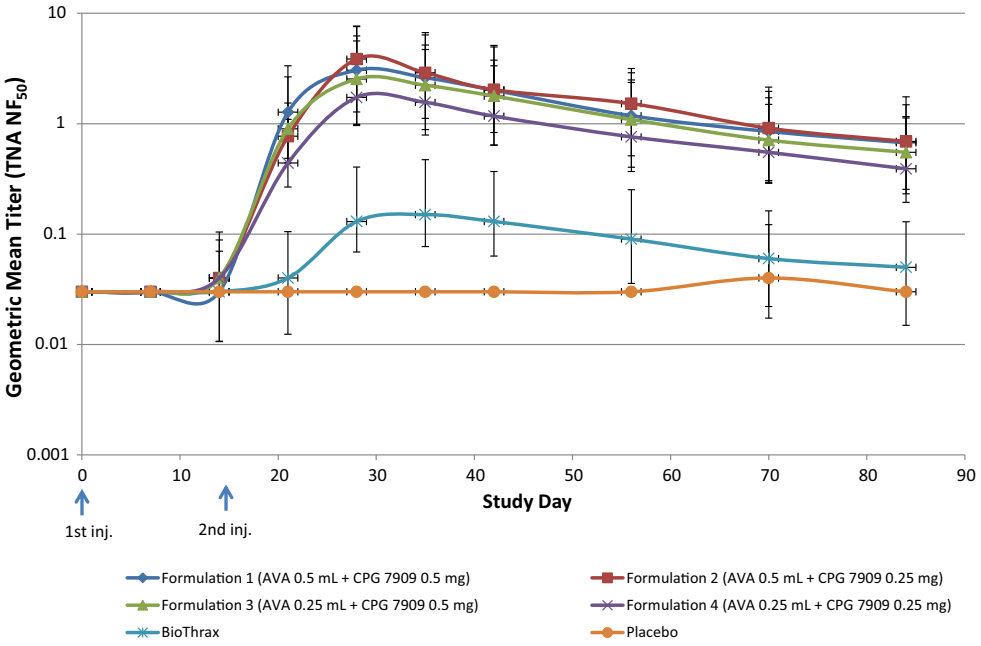

Fig. 3. Anthrax toxin neutralizing antibody titers. Blood samples for measurement of toxin neutralizing antibody titers (TNA NF_{50}) were collected on Days 0 (pre-injection), 7, 14 (pre-injection), 21, 28, 35, 42, 56, 70, and 84. Geometric mean and 95% CIs are shown for each study day. TNA = toxin neutralizing antibody; NF_{50} = 50% neutralization factor.

3.2.3. C-reactive protein

CRP levels peaked 2 days post-immunization in the active treatment groups, and returned to baseline levels by Days 7 and 14. On Day 2, the mean values were higher in the AV7909 groups vs. the BioThrax group and were independent of CPG 7909 dose: AV7909 Formulation 1 (15.9 mg/L), Formulation 2 (8.4 mg/L), Formulation 3 (7.7 mg/L), and Formulation 4 (7.9 mg/L). CRP levels for BioThrax and saline were 3.6 mg/L and 1.3 mg/L, respectively.

3.3. Immunogenicity

3.3.1. TNA NF_{50} titers

Five subjects (2 in the BioThrax group, and 1 in each of the AV7909 Formulation 1–3 groups) were excluded from the immunogenicity population because they did not receive the second injection.

After 2 doses, TNA NF_{50} GMTs in the AV7909 groups were higher than in the BioThrax group (Fig. 3). AV7909 Formulation 2 elicited the highest peak response followed by AV7909 Formulation 1, although differences in GMTs among the 4 AV7909 groups were not statistically significant on Days 21, 28, 35, 42, 56, 70, or 84. In the BioThrax group, the TNA NF_{50} GMT peaked on Day 35 (0.15). Peak GMTs in the AV7909 Formulation 1–4 groups (3.05, 3.85, 2.54, and 1.73, respectively) were achieved on Day 28, 2 weeks after the second injection. On Day 84, GMTs in the AV7909 groups remained higher than the corresponding value in the BioThrax group (0.39–0.69 vs. 0.05, respectively).

4. Discussion

In order to identify an optimal combination of AVA and CPG 7909 that maintained the increased and accelerated immunity seen in a previous Phase 1 study [1], but did not increase systemic reactogenicity, the current study was designed to evaluate each of 2 different doses of AVA (0.5 mL and 0.25 mL) preformulated with each of 2 different doses of CPG 7909 (0.5 mg and 0.25 mg) in 4 AV7909 formulations. The doses of BioThrax and CPG 7909 used in a previous Phase 1 study were 0.5 mL and 1.0 mg, respectively [1]. All investigational products were administered IM, the currently licensed route of administration for BioThrax for general use prophylaxis [5].

Intramuscular administration of 2 doses of each of the 4 formulations of AV7909 resulted in increases in peak TNA response compared with IM administration of 2 doses of BioThrax. It is important to note that BioThrax is currently indicated for general use prophylaxis as an IM injection (0, 1, and 6 months with booster doses at 12 and 18 months and at 1 year intervals thereafter).

Among the AV7909 formulations, Formulation 2 yielded the highest peak TNA response followed by Formulation 1, although differences in peak GMTs among the 4 AV7909 groups were not statistically significant. Large variability was noted in individual TNA responses to AV7909, suggesting that larger group sizes are needed to detect small potential differences among the 4 AV7909 formulations. TNA responses peaked earlier with AV7909 (Day 28, 2 weeks after the second vaccination) than with BioThrax (Day 35, 3 weeks after the second vaccination). TNA NF_{50} GMTs in each AV7909 group remained higher than the corresponding value in the BioThrax group at the final time point tested (Day 84). In a previous Phase 1 study of BioThrax alone, BioThrax + CPG 7909, and CPG 7909 alone administered IM on Days 0, 14, and 28, the peak TNA concentration in both active treatment groups was achieved at 2 weeks after the third vaccination [1]. Peak TNA titers were higher in the BioThrax + CPG 7909 group vs. the BioThrax group.

One SAE occurred during the first 84 days of the study (elevated hepatic enzymes), and 1 SAE (glioblastoma multiforme) and 1 death (motor vehicle accident) were reported during the 6- and 12-month follow-up telephone calls, respectively. These events were assessed as not related to vaccination.

The number of subjects who received an AV7909 formulation and had a severe related AE was less than or equal to the number of subjects who received BioThrax and had a severe related AE. Injection site reaction was the most common related AE occurring in 73.7–100% of subjects in each active treatment group. Although the study was not powered to evaluate both incidence and severity of AEs, all AV7909 formulations except for Formulation 2 appeared to have a greater percentage of subjects with injections site

reactions assessed as moderate or severe compared with BioThrax alone (Fig. 2).

Transient lymphopenia (24 h in duration) occurred after the first dose in each AV7909 group, but not in the BioThrax or saline placebo group. Transient lymphopenia and/or transient leukopenia have been reported in other clinical trials with CPG 7909 alone [9] or with CPG 7909 as a vaccine adjuvant [10–12]. CPG oligonucleotides have been shown to cause a dendritic cell-mediated chemokine response resulting in T-cell migration to the peripheral tissues [13]. Since the lymphopenia observed in response to administration of AV7909 may be the result of altered lymphocyte trafficking, and the event is of short duration, it is unlikely to affect susceptibility to infections in healthy or special populations.

CRP levels peaked 2 days after the first immunization in the active treatment groups, and returned to baseline levels by Days 7 and 14. On Day 2, mean values ranged from 7.9 mg/L to 15.9 mg/L (highest with Formulation 1) in the AV7909 groups vs. 3.6 mg/L in the BioThrax group. This finding is in agreement with the results of a study where 2 SC doses of CPG 7909 (0.0025–0.08 mg/kg) were administered 14 days apart. In that study, CRP levels increased in a dose-dependent manner and peaked at approximately 48 h after each dose. Mean peak values were up to 20 mg/L after the first dose and up to 15 mg/L after the second dose [9].

The injection site reaction with highest subject incidence after each dose was pain at the injection site and arm motion limitation. This observation agrees with the reactogenicity data reported in a previous Phase 1 study [1], which combined data from in-clinic examinations, subject diary cards, and subject interviews. Pain at the injection site (98.5%) and arm motion limitation (88.4%) were the most common injection site reactions in the BioThrax + CPG 7909 group.

We report here for the AV7909 Formulation 1 group after the first and second injections, respectively, subject incidences of headache (61.1%, 38.9%), muscle ache (44.4%, 50.0%), and fatigue (50.0%, 44.4%). Moreover, the percentage of subject with moderate or severe systemic reactions in the Formulation 1 group was higher than in any other dosing group. Combined systemic reactogenicity data in a previous Phase 1 study [1] showed that 69.6% of subjects who received BioThrax + CPG 7909 had headache, 63.7% had muscle ache, and 62.3% had fatigue.

5. Conclusions

Compared with BioThrax, each AV7909 formulation elicited a higher and accelerated TNA response after IM administration of 2 doses 2 weeks apart. There was no significant difference in the immunogenicity of the 4 AV7909 formulations. The incidences of local and systemic reactions reported here with lower doses of CPG 7909 were lower than reported in a previous Phase 1 study in which BioThrax was mixed with a higher, 1 mg dose of CPG 7909 [1]. Further studies of doses and dosing regimens are planned to assess the immunogenicity and reactogenicity of AV7909.

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