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Randomized, double-blind, active-controlled study evaluating the safety and immunogenicity of three vaccination schedules and two dose levels of AV7909 vaccine for anthrax post-exposure prophylaxis in healthy adults

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ABSTRACT

AV7909 vaccine being developed for post-exposure prophylaxis of anthrax disease may require fewer vaccinations and reduced amount of antigen to achieve an accelerated immune response over BioThrax® (Anthrax Vaccine Adsorbed).

A phase 2, randomized, double-blind, BioThrax vacccine-controlled study was conducted to evaluate the safety and immunogenicity of three intramuscular vaccination schedules and two dose levels of AV7909 in 168 healthy adults. Subjects were randomized at a 4:3:2:4:2 ratio to 5 groups: (1) AV7909 on Days 0/14; (2) AV7909 on Days 0/28; (3) AV7909 on Days 0/14/28; (4) half dose AV7909 on Days 0/14/28; and (5) BioThrax vaccine on Days 0/14/28.

Vaccinations in all groups were well tolerated. The incidences of adverse events (AEs) were 79% for AV7909 subjects and 65% for BioThrax subjects; 92% of AV7909 subjects and 87% of BioThrax subjects having AEs reported Grade 1–2 AEs. No serious AEs were assessed as potentially vaccine-related, and no AEs of potential autoimmune etiology were reported. There was no discernible pattern indicative of a safety concern across groups in the incidence or severity of reactogenicity events.

Groups 2–4 achieved success for the primary endpoint, demonstrated by a lower 95% confidence limit of the percentage of subjects with protective toxin neutralizing antibody NF_{50} values ($\geq$0.56) to be $\geq$40% at Day 63. Group 1 marginally missed the criterion (lower bound 95% confidence limit of 39.5%). Immune responses were above this threshold for Groups 1, 3 and 4 at Day 28 and all groups at Day 42.

Further study of an AV7909 two-dose schedule given 2 weeks apart is warranted in light of the favorable tolerability profile and immunogenicity response relative to three doses of BioThrax vaccine, as well as preliminary data from nonclinical studies indicating similar immune responses correlate with higher survival for AV7909 than BioThrax vaccine.

1. Introduction

The combination of BioThrax® (Anthrax Vaccine Adsorbed [AVA]) and CPG 7909 (a synthetic immunostimulatory oligonucleotide), known as AV7909, is being clinically investigated for its potential to achieve an accelerated immune response for protection against anthrax disease in a post-exposure prophylaxis (PEP) setting. A 3-dose series of BioThrax vaccine administered subcutaneously (SC) at 0, 2, and 4 weeks alongside a 60-day antimicrobial regimen is approved by the United States (US) Food and Drug Administration (FDA) and recommended by the Advisory Committee on Immunization Practices for PEP of anthrax [1]. Compliance with the antimicrobial regimen has been problematic: less than 50% of exposed individuals were compliant following the 2001 anthrax attacks in the US [2]. An enhanced anthrax vaccine including CPG 7909 may require fewer vaccine doses and reduced amount of antigen, potentially promoting compliance with the

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full vaccine schedule while providing a greater probability of survival. Earlier protection may reduce the duration of antimicrobials required.

Two phase 1 trials exploring the AV7909 vaccine have been completed in 174 healthy adults. In the initial trial, an admixture prior to injection of 0.5 mL BioThrax vaccine + 1 mg CPG 7909 significantly increased serum levels of anti-protective antigen (PA) antibody and toxin neutralizing antibody (TNA) compared with BioThrax vaccine alone, including at a much earlier time point, by at least 3 weeks [3]. Vaccination was well tolerated with a trend toward increased frequency and severity of local and systemic reactions in the BioThrax vaccine + CPG 7909 group. The second trial identified a formulation of AV7909 for further clinical development (0.5 mL BioThrax vaccine + 0.25 mg CPG 7909) that produced an enhanced immune response without increased reactogenicity [4]. This formulation was evaluated in the phase 2 trial described here.

The primary objectives of the current phase 2 trial were to assess safety of the selected AV7909 formulation and assess immunogenicity measured as TNA 50% neutralization factor (NF_{50}) at Day 63. As a secondary objective, immunogenicity at earlier time points was also assessed (Days 28, 42).

2. Materials and methods

2.1. Investigational products

All vaccines were administered intramuscularly (IM) in the deltoid muscle using a 25-ga sterile needle and 1 cm^3 syringe. Alternating arms were used for each successive injection. Refrigeration within the range of 2–8 °C was required for the AV7909 and AVA vaccines.

AVA, manufactured by Emergent BioSolutions Inc. (Lansing, Michigan, US) and previously described [4], is a sterile, milky-white suspension made from cell-free filtrates of microaerophilic cultures of an avirulent, nonencapsulated strain of *Bacillus anthracis*. Commercial lot FAV392A was supplied for the study. A single vaccine dose was 0.5 mL.

AV7909 drug product, a pre-formulated, sterile, milky-white suspension manufactured by Par Pharmaceutical (Rochester, Michigan, US), is composed of AVA bulk drug substance and CPG 7909 adjuvant. Approximately 6.2 mL of AV7909 vaccine was supplied in glass vials as a multidose final drug product. Each full dose contained 0.5 mL AVA + 0.25 mg CPG 7909 and each half-dose contained 0.25 mL AVA + 0.125 mg CPG 7909. Lot TC2994 was used in the study.

Placebo of 0.5 mL of sterile, colorless, preservative-free saline for injection (0.9% sodium chloride) was administered in Groups 1 and 2 to mask the dosing schedule.

2.2. Study design

This was a phase 2, multicenter, randomized, parallel-group, active-controlled, double-blind study evaluating the safety and immunogenicity of AV7909 for PEP of anthrax disease in 168 subjects recruited at four clinical research centers in the US. Eligible subjects were aged 18–50 years and in general good health, having no previous history of exposure to anthrax or anthrax vaccine and no chronic conditions or exposure to products that may have biased an evaluation of the immune response.

Subjects were allocated to treatment groups according to a computer-generated randomization list prepared by an independent, unblinded statistician. An unblinded site staff member accessed the interactive web response system to receive a printout with the treatment assignment, which was subsequently secured in a limited-access area to prevent accidental unblinding. Subjects were randomized using a 4:3:2:4:2 ratio in blocks of 15 to 1 of 5 groups comprising 3 IM vaccination schedules and 2 dose levels: (1) full dose AV7909 on Days 0/14; (2) full dose AV7909 on Days 0/28; (3) full dose AV7909 on Days 0/14/28; (4) half dose AV7909 on Days 0/14/28; and (5) full dose BioThrax vaccine on Days 0/14/28. Randomization was stratified to ensure at least 40% of subjects per group were of each gender and that equal proportions of subjects were between 18–30 or 31–50 years of age.

Procedures were instituted to ensure investigators, all staff performing subject assessments, and all subjects remained blinded to treatment assignment. Vaccine preparation and administration were performed by unblinded site personnel. All syringes were covered during vaccine administration, leaving only the hub exposed.

The BioThrax immunization regimen used in this study has been shown to achieve circulating TNA titers that have conferred at least 70% survival to rabbits and non-human primates after *B. anthracis* spore challenge [5,6].

The trial was conducted in accordance with Good Clinical Practice and ethical principles that have their origin in the Declaration of Helsinki. Informed consent was obtained from subjects at Screening after informing them about the study and associated risks. Safety oversight was supplemented by an independent safety monitoring committee (SMC) consisting of 3 physicians, and also independent safety monitors (ISMs). Interim safety data were reviewed by the SMC after 50 subjects and 100 subjects had completed the Day 14 visit. No safety concerns were identified during these reviews.

2.3. Immunogenicity assessment

Serum samples for determination of immunogenicity were collected on Days 0 (before vaccination), 21, 28 (before vaccination), 35, 42, 49, 63, and 84. Samples were tested using a validated, high-throughput, cell-based bioassay by Battelle Memorial Institute (West Jefferson, Ohio, US) to measure neutralization of anthrax lethal toxin (LT) by antibodies generated in response to vaccination. An NF_{50} value was calculated as the ratio of effective dose that provided 50% neutralization (ED_{50}) of the human test sample to ED_{50} of a human reference serum from subjects vaccinated with BioThrax vaccine. The reference standard, AVR801, was the same used in the prior clinical studies of AV7909 [3,4].

The primary immunogenicity outcome was the lower bound of the 95% conference interval (CI) for the proportion of subjects in each group with Day 63 NF_{50} values $\geq$0.56, the threshold of 70% protection established for BioThrax vaccine. Secondary outcomes also focused on an evaluation of immunogenicity based on proportion of subjects meeting or exceeding the NF_{50} threshold of protection at earlier time points (at Day 28 in Groups 1, 3, and 4; at Day 42 in all groups).

2.4. Safety assessment

Subjects were evaluated for safety at clinic visits from Day 0 to Day 84 and also by phone contacts occurring 6 months and 12 months after the last vaccination.

Safety was assessed through Day 84 by clinical laboratory test results graded against a modified version of the FDA's toxicity grading scale [7], monitoring of adverse events (AEs; including serious AEs [SAEs]; and AEs of special interest [AESI], defined as having a potential autoimmune etiology), vital signs, and physical examinations. Serum samples were collected on Days 0 (before vaccination), 42, and 84 for autoantibody testing (anti-nuclear antibodies and rheumatoid factor) if any subjects reported AESIs during the study. Notably, AEs were recorded for Grade 3 or higher laboratory test abnormalities, any clinical laboratory or vitals sign abnormalities considered clinically significant by the investigator, and any new or

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