

The effect of raxibacumab on the immunogenicity of anthrax vaccine adsorbed: a Phase IV, randomised, open-label, parallel-group, non-inferiority study

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Abstract

Background

Raxibacumab is a monoclonal antibody (Ab) which binds protective antigen (PA) of *Bacillus anthracis* and is approved for treatment and post-exposure prophylaxis (PEP) of inhalational anthrax. Anthrax vaccine adsorbed (AVA), for anthrax prophylaxis, consists primarily of adsorbed PA. This post-approval study evaluated the effect of raxibacumab on immunogenicity of AVA.

Methods

In this open-label, parallel-group, non-inferiority study in three centres in the USA, healthy volunteers (aged 18–65 years) with no evidence of PA pre-exposure were randomised 1:1 to receive either subcutaneous 0.5 mL AVA on Days 1, 15, and 29 or raxibacumab intravenous infusion (40 mg/kg) immediately before AVA on Day 1, followed by AVA only on Days 15 and 29. It was an open-label study to investigators and participants, however, the sponsor remained blinded during the study. The primary outcome was ratio of geometric mean concentration (GMC) of anti-PA Ab between the groups 4 weeks after the first AVA dose in the per-protocol (PP) population. The non-inferiority margin for the ratio of GMC was pre-defined as the upper limit of the 90% confidence interval (CI) <1.5. Toxin-neutralising activity, safety and raxibacumab pharmacokinetics were also assessed. ClinicalTrials.gov registration number NCT02339155.

Findings

Between 24 February, 2015 and 6 June, 2017, a total of 873 participants were screened and 573 were randomised in the study. At Week 4, in the PP population, the anti-PA Ab GMC ratio of the AVA group (26.5 µg/mL, 95% CI: 23.6, 29.8, N=276) to the AVA plus raxibacumab group (22.5 µg/mL, 95% CI: 20.1, 25.1, N=269) was 1.18 (90% CI: 1.03, 1.35,

p-value=0.0019), which met the pre-defined non-inferiority margin (upper limit of 90% CI <1.5). As a secondary outcome, adverse events (AEs) in the safety population were similar across groups (AVA: N=87/286, 30.4%; AVA plus raxibacumab: N=80/280, 28.6%) and no treatment-related serious AEs were reported.

Interpretation

Co-administration of raxibacumab with AVA does not negatively impact AVA immunogenicity. This suggests that combining raxibacumab with AVA may provide added benefit in PEP against inhalational anthrax.

Funding

US Biomedical Advanced Research and Development Authority (BARDA) and GlaxoSmithKline (GSK).

Research in context

Evidence before this study

This study arose as a Phase IV, post-approval commitment study from the US Food and Drug Administration following the granting of marketing authorisation for raxibacumab in the USA in 2012. Published data in rabbits had indicated that co-administration of anthrax vaccine adsorbed (AVA) with polyclonal immunoglobulin (Ig) derived from humans vaccinated with AVA abrogated the immune response to AVA. No other animal or human data was available examining the interaction between anthrax vaccine and anthrax specific Ig. We searched PubMed for articles between database inception and 27 December, 2019 using the terms “anthrax vaccine / AVA”, “raxibacumab / anthrax monoclonal”, “immunoglobulin”, which we then reviewed for relevance. We did not apply any language restrictions.

Added value of this study

Current US Center for Disease Control and Prevention (CDC) guidelines recommend the use of a prolonged 42-day course of antibiotics (60 days for immunocompromised persons) and post-exposure vaccination following potential exposure to anthrax via the inhalational route. Administration of anti-toxin therapy (such as raxibacumab) is recommended by the US CDC for any patient in which there is a high clinical suspicion of systemic anthrax. Prolonged course of antibiotics may be hampered by poor compliance, especially in asymptomatic individuals in the post-exposure setting. Additionally, in some circumstances antibiotic use may not be appropriate. Prior to this study, there has not been data to support the administration of anti-toxin therapy at the same time as post-exposure vaccination. However, this approach has an advantage; the duration of protection from a single dose of anti-toxin therapy will cover the period during the development of the protective immune response to

vaccination. The findings from the current study indicate that administration of raxibacumab with AVA does not negatively impact the immunogenicity of AVA or the pharmacokinetic profile of raxibacumab or result in any new safety findings.

Implications of all the available evidence

In the context of available evidence, the study provides an additional or alternative post-exposure prophylaxis regimen against inhalational anthrax. The prolonged antibiotic regimen for post-exposure prophylaxis is challenging, and in certain circumstances antibiotics may be contraindicated for some patients or infection may be caused by antibiotic-resistant strains. In these instances, administration of raxibacumab or other anthrax-specific anti-toxins, which have a long half-life with single dose administration and specifically target the toxemia, may be a more suitable option.

Introduction

Anthrax is a rare zoonotic infection caused by the Gram-positive, aerobic, spore-forming bacteria *Bacillus anthracis*. Inhalational anthrax is particularly deadly, and inhalation of aerosolised *B. anthracis* spores into the lower respiratory tract is likely to account for the highest rates of morbidity and mortality after use of anthrax as a biological weapon.¹ In September 2001, following the deliberate sending of anthrax spores via the US postal service, five of the 11 people with inhalational anthrax died (45% mortality), in spite of best available treatment with antibiotics and supportive care.² This high mortality rate led to development of therapies specifically targeting *B. anthracis* toxins, which are responsible for disease pathophysiology.

Following inhalation, spores germinate, resulting in bacteraemia and toxemia. Protective antigen (PA) binds to receptors on host cells and after proteolytic cleavage forms multimers which bind to edema factor or lethal factor with high affinity; this leads to the formation of edema toxin and lethal toxin, respectively. These assembled toxins are translocated across the cell membrane into the cytosol where they exert their toxic effects and have a downstream impact on host defences, which are responsible for severe systemic disease.^{1,3} Early diagnosis and initiation of appropriate treatment are critical to improving survival.⁴ Guidelines from the US Centers for Disease Control and Prevention (CDC) and from the European Union recommend 60 consecutive days of antibiotic treatment for inhalational anthrax to ensure clearance of germinating spores.^{4,5} The CDC guidance also recommends that anti-toxin therapy may be added if there is a high level of clinical suspicion of systemic anthrax.⁴

Similarly, for exposed individuals who are at risk of anthrax infection, timely and appropriate post-exposure prophylaxis (PEP) is life-saving. A prolonged antibiotic course (ciprofloxacin or doxycycline) combined with anthrax vaccine is recommended by the CDC.^{4,6} The only

approved anthrax vaccine in the USA is anthrax vaccine adsorbed (AVA), which is mainly composed of adsorbed PA. Vaccines require multiple injections over several weeks before immunity is initially established,^{7,8} so may not be effective in the event of acute exposure to *B. anthracis*.

Raxibacumab is a fully human monoclonal immunoglobulin (Ig) G1 λ antibody (Ab) that binds the PA component of anthrax toxin and prevents PA from binding to the host cell surface, thus blocking the formation and effects of edema toxin and lethal toxin. Raxibacumab was developed in response to the anthrax event of 2001 and was approved by the US Food and Drug Administration in 2012 for the treatment of inhalational anthrax in combination with antimicrobial drugs and for prophylaxis of inhalational anthrax when alternative therapies are unavailable or inappropriate. Raxibacumab was the first biologic drug approved under the Animal Rule,⁹ based on efficacy studies in animal models of inhalational anthrax, and safety and pharmacokinetic (PK) studies in healthy human volunteers.¹⁰ The prolonged antibiotic regimen for PEP is challenging, and in certain circumstances antibiotics may not be appropriate. In these instances, administration of raxibacumab or other anthrax-specific anti-toxins, which have a long half-life with single-dose administration and specifically target the toxemia, may be a more suitable therapeutic option.

Based on animal data in which polyclonal anthrax Ig from human AVA vaccine recipients co-administered with AVA abrogated the immune response in rabbits,¹¹ there was concern that co-administration of AVA with raxibacumab as part of a PEP regimen, could potentially result in raxibacumab binding the PA component of AVA; this could lead to reduced vaccine immunogenicity and efficacy, with decreased anti-PA Ab concentrations and toxin-neutralising activity (TNA) titres. As a post-approval commitment following licensure of

raxibacumab, this study was designed to investigate the effect of raxibacumab on the immunogenicity of AVA in healthy human volunteers.

Methods

Study design and participants

This was a Phase IV, randomised, open-label, parallel-group study, conducted at three centres in the USA between February 2015 and June 2017. The trial was conducted in accordance with Good Clinical Practice guidelines and the provisions of the Declaration of Helsinki.^{12,13} The trial protocol, available at ClinicalTrials.gov, was approved by institutional review boards, and all subjects provided written informed consent. The protocol was amended once on 25 April, 2016, to update contraception requirements. Anonymised individual participant data and study documents can be requested for further research from www.clinicalstudydatarequest.com.

Participants eligible for enrolment in the study were men or women, aged 18–65 years, who were assessed as healthy based on medical history, physical examination, and laboratory tests. Women were only included if they were not pregnant, breastfeeding or, if sexually active, were willing to use adequate contraception during the study. Participants were excluded if they had been previously vaccinated against PA, had an anti-PA Ab concentration greater than 2 times the lower limit of quantitation, or were a member of the military, a laboratory worker, first responder, health care worker, or those who would otherwise be at higher risk of exposure to anthrax. The other main exclusion criteria were participants who had a suspected or confirmed immunosuppressive condition, or were receiving immunosuppressive/cytotoxic therapy, or had received long-acting immune-modifying drugs or chronically administered (defined as >14 consecutive days) systemic immunosuppressants

or had been administered non-study-related Igs, blood products, or vaccines. A complete list of exclusion criteria is available in the Appendix, page 2.

Randomisation and masking

Participants with no evidence of pre-exposure to PA were randomised 1:1 to receive either AVA alone or an intravenous (IV) infusion of raxibacumab immediately before the AVA dose on Day 1. Participants were assigned to treatment groups according to a pre-generated balanced independent randomisation schedule. A set of randomisation envelopes (one per subject) were generated and a randomisation envelope for each subject was opened prior to dosing to reveal the individual's treatment assignment. This was an open-label study and investigators, site staff, and subjects had knowledge of individual study treatments at randomisation. However, to minimise bias, specific measures were taken during the study. The sponsor (GSK) team were blinded to individual subject's randomisation and only the site pharmacy had access to the full randomisation schedule. Three sequential cohorts with interim analyses after each of the first two cohorts were planned, with an independent statistician and clinician involved in performing the interim analyses. After each interim analysis, communication with the sponsor was limited to whether the pre-defined futility boundary was crossed; no summary of the data was provided to the sponsor. The trial enrolled all three cohorts because none of the prospective criteria for trial discontinuation was met at any of these interim analyses (Appendix, page 5).

Procedures

Study treatments were supplied as raxibacumab 50 mg/mL for injection (GSK, Rockville, MD, USA), AVA 0.5 mL dose (BioThrax, Emergent BioSolutions Inc, Lansing, MI, USA) and diphenhydramine 25–50 mg. Diphenhydramine and AVA were both commercially sourced.

Participants received either subcutaneous (SC) AVA 0.5 mL on Days 1, 15, and 29 (AVA group), or raxibacumab IV infusion (40 mg/kg) immediately before AVA 0.5 mL SC on Day 1, followed by AVA 0.5 mL SC only on Days 15 and 29 (AVA plus raxibacumab group). Raxibacumab and AVA were administered according to their respective approved product labels;^{14,15} all subjects in the AVA plus raxibacumab group were pre-administered diphenhydramine 25–50 mg within 60 minutes prior to the raxibacumab infusion to reduce the risk of infusion reactions as per the raxibacumab label.

Immunogenicity of AVA and seroconversion were assessed on Days 29 (Week 4), Day 57 (Week 8), and at a follow-up visit on Day 183 (Week 26). The study duration was selected because it allowed for assessment of the durable immune response to AVA. Safety assessments were performed throughout the study. Blood samples for PK assessments were also collected from Day 1 (0, 30 min, 2–6 h) and on all subsequent study visits (Days 15, 29, 57, and 183) in the AVA plus raxibacumab group. The study design is outlined in the Appendix, Figure S1.

Endogenous anti-PA Ab was measured by electrochemiluminescent immunoassay following immunodepletion of raxibacumab from the serum using a mouse anti-raxibacumab monoclonal Ab (mAb). TNA was assessed prior to immunodepletion of raxibacumab using a cell viability assay measuring inhibition of lethal toxin-mediated cell cytotoxicity that occurs when PA activity is neutralised. Serum raxibacumab concentrations were determined by ultra-high-pressure liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) analysis on trypsin-digested samples following immunocapture purification with a mouse-anti-raxibacumab mAb. Further details of assays, including the bioanalysis of raxibacumab are provided in the Appendix, page 3-4.

Outcomes

The objective of the study was to demonstrate that the immunogenicity of AVA plus raxibacumab was non-inferior to AVA alone. The primary outcome was immunogenicity of AVA at Week 4, assessed by comparing the ratio of geometric mean concentrations (GMCs) of anti-PA Ab 28 days after the first AVA dose, between the AVA and AVA plus raxibacumab groups. Secondary outcomes were immunogenicity of AVA at Weeks 8 and 26, and seroconversion, defined as a ≥ 4 -fold increase in TNA titre from baseline at Weeks 4, 8, and 26. Safety assessments included frequency of reported adverse events (AEs), review of vital signs, urinalysis, and clinical laboratory data. Raxibacumab PK parameters were also evaluated.

Statistical analysis

The sample size of 534 evaluable subjects was calculated assuming 1:1 randomisation and two interim analyses for futility after 30 and 100 evaluable subjects. The sample size was computed assuming \log_{10} transformed data with difference in means of 0.176 [$\log_{10}(1.5)$] and standard deviation of 0.75 to obtain power of 80% with one-sided type 1 error rate of 5% to demonstrate the primary objective of non-inferiority between AVA and AVA plus raxibacumab groups. The non-inferiority test was based on the confidence interval (CI) for the ratio of anti-PA Abs (attributable to immune response to AVA) GMCs between AVA and AVA plus raxibacumab groups at Week 4. The pre-defined non-inferiority margin for the ratio of geometric means was < 1.5 for the upper limit of the 90% CI.¹⁶ For the secondary outcome, the percentage of subjects who seroconverted (≥ 4 -fold increase in TNA titre from baseline) was summarised at Weeks 4, 8, and 26.

The primary analysis was based on the per-protocol (PP) population, which comprised all evaluable subjects who received correct treatment within the protocol-specified visit window

and completed the primary study outcome assessment, without a protocol deviation requiring exclusion from the population. The intent-to-treat (ITT) population comprised all randomised subjects. The safety analysis was based on the safety population which comprised all randomised subjects receiving ≥ 1 dose of study treatment. The PK population comprised all evaluable subjects who received raxibacumab and who had at least one post-dose concentration assessment at a scheduled PK time point. Further details on statistical analyses are in the Appendix, page 5-6.

Role of the funding source

GSK (the study sponsor) had a role in study design, data collection, data analysis, data interpretation, and writing of the report. US Biomedical Advanced Research and Development Authority (BARDA) reviewed the clinical study report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between 24 February, 2015 and 6 June, 2017, a total of 873 subjects were screened and 573 were randomised to the AVA (n=287) or the AVA plus raxibacumab (n=286) group; 537 participants completed the study as planned (AVA: n=272; AVA plus raxibacumab: n=265). Baseline characteristics were well balanced between treatment groups (Table 1) and approximately 50% of participants in each of the treatment groups was female. Concomitant medications that had been agreed by the study investigators were permitted during the study and were not considered to affect the interpretation of the study results. The most commonly used concomitant medications in both treatment arms were ethinyloestradiol, paracetamol, and ibuprofen (Appendix, Table S1). The most common reason for withdrawal from the

study and/or treatment was loss to follow-up. At study end, the PP, safety, and PK populations comprised 545, 566, and 279 subjects, respectively (Figure 1).

Immunological non-inferiority of AVA plus raxibacumab versus AVA alone was demonstrated in the PP population based on the ratio of anti-PA Ab GMC between the two groups at Week 4 (AVA plus raxibacumab: 22.5 µg/mL, 95% CI: 20.1, 25.1, N=269; AVA: 26.5 µg/mL, 95% CI: 23.6, 29.8, N=276); the upper limit of the 90% CIs for the ratio of anti-PA Ab GMC between the AVA and AVA plus raxibacumab group was 1.35, which met the pre-specified criterion of <1.5 (Figure 2). Sensitivity analysis using two methods of imputation for subjects without Week 4 anti-PA Ab concentration results also established non-inferiority between treatment groups in the ITT population (Appendix, Table S2). Furthermore, anti-PA Ab GMCs in the PP population were comparable at Week 8 (AVA plus raxibacumab: 46.1 µg/mL, 95% CI: 41.9, 50.8, N=267; AVA: 50.5 µg/mL, 95% CI: 46.1, 55.2, N=275) and Week 26 (AVA plus raxibacumab: 10.2 µg/mL, 95% CI: 9.48, 11.0, N=258; AVA: 10.0 µg/mL, 95% CI: 9.24, 10.8, N=267) (Figure 2). One subject (1/276, 0.4%) who received AVA alone and six subjects (6/269, 2.2%) who were co-administered AVA plus raxibacumab did not mount anti-PA responses at Weeks 4, 8, or 26.

TNA response is a functional correlate of the anti-PA response. At Weeks 4 and 8 in the PP population, the serum TNA geometric mean titres (GMTs) in subjects from the AVA plus raxibacumab group was 1110 (95% CI: 1030, 1210, N=269) at Week 4 and 830 (95% CI: 763, 904, N=266) at Week 8, respectively, which were higher than those of the AVA group at 328 (95% CI: 276, 388, N=274) at Week 4 and 609 (95% CI: 541, 686, N=273) at Week 8, respectively. Whereas at Week 26, these titres were comparable (AVA plus raxibacumab: 72.3, 95% CI: 66.3, 78.8, N=257; AVA: 70.3, 95% CI: 62.9, 78.5, N=264) (Figure 3). The statistical summary demonstrates that the percentage of subjects who achieved TNA seroconversion at Week 4 in the PP population was higher in the AVA plus raxibacumab

group (N=267/269; 99.3%, 95% CI: 97.3, 99.8) than the AVA group (N=211/276; 76.4%, 95% CI: 71.1, 81.1). The percentage of subjects who achieved seroconversion at Week 8 (AVA plus raxibacumab: N=264/269, 98.1%, 95% CI: 96.7, 99.6; AVA: N=263/276, 95.3%, 95% CI: 92.5, 97.5) and Week 26 (AVA plus raxibacumab: N=86/269, 32.0%, 95% CI: 27.9, 39.3; AVA: N=88/276, 31.9%, 95% CI: 27.6, 38.8) was comparable in both treatment groups (Figure 3).

The number of subjects in the safety population who experienced one or more AEs that emerged during treatment was similar in the AVA (87/286, 30.4%) and AVA plus raxibacumab (80/280, 28.6%) groups (Table 2). Injection-site reactions and headache were the most commonly reported non-serious AEs that emerged during treatment. In the AVA group, five subjects experienced serious AEs (SAEs), including one death from illicit drug overdose. In the AVA plus raxibacumab group, three subjects experienced SAEs, including one death from suicide (Appendix, Table S3). None of these SAEs or deaths in either group was considered by the study investigator to be treatment-related.

There were six subjects in the AVA plus raxibacumab group who experienced AEs related to infusion of raxibacumab, which were not serious. These subjects were withdrawn from the study and did not receive the AVA dose on study Day 1. One subject in the AVA group was withdrawn from the study due to a moderate headache prior to the first AVA dose. There were no clinically important changes in laboratory values across treatment groups.

Three subjects became pregnant during the study, all in the AVA group. Two pregnancies ended in first trimester spontaneous abortions with no apparent congenital anomaly. One subject received AVA at Week 1 of pregnancy and had a spontaneous abortion 83 days after the first dose. The other subject became pregnant approximately 105 days after first AVA dose and had a spontaneous abortion 191 days after the first dose. Neither was attributed to

study drug by investigators and both had other potential causes. There was one healthy live birth at 39 weeks gestation which occurred 434 days after the first dose of AVA.

Raxibacumab exposure levels, assessed as area under the serum concentration versus time curve, maximum concentration, and half-life, were similar to those reported in previous clinical studies after administration of IV raxibacumab (40 mg/kg) alone or in combination with ciprofloxacin in healthy human volunteers (Figure 4).¹⁰ Additional PK parameters are summarised in the Appendix, Table S4.

Discussion

The use of anthrax as a biological weapon remains a global threat. The likely route of infection with anthrax spores during deliberate release is via inhalation which has a mortality rate of 45–54% even with antibiotic treatment.^{2,17} Vaccination for PEP is recommended to prevent disease following exposure to bacterial spores.⁴ Filtered supernatants from bacterial cell cultures have been used as anthrax vaccines for over 60 years. AVA is the only approved anthrax vaccine in the USA and Anthrax vaccine precipitated (AVP) is licensed in the UK. Studies have demonstrated that the main protein component of the AVA and AVP vaccines is PA and that Abs generated against PA through vaccination can protect susceptible animals from the inhalation form of anthrax (reviewed in¹⁸). Raxibacumab is also indicated for PEP for inhalational anthrax, and could potentially be co-administered with anthrax vaccines such as AVA; however, co-administration is not currently in prescribing information of either product. Because the primary antigen in AVA is PA protein, it was hypothesised that raxibacumab may bind PA from AVA, leading to decreased anti-PA Ab concentrations, TNA titres, and vaccine efficacy. In addition, a study in rabbits, in which polyclonal anthrax Ig (purified from plasma of human recipients of the AVA vaccine) co-administered with AVA abrogated the immune response¹¹. Results from our study, which is one of the largest

prospective, mAb-vaccine interaction studies of its kind, refuted this hypothesis. Previous studies have assessed interactions between vaccines and concomitant polyclonal Ig in other infectious diseases. For example, immunological interference was demonstrated with hepatitis A,¹⁹ but not with polio or hepatitis B;^{20,21} indeed, hepatitis B-specific Ig and vaccination are recommended for concomitant administration at birth in high-risk populations.²²

In our study, primary immunogenicity analyses met the pre-defined non-inferiority margin in both PP and ITT populations, confirming that AVA co-administered with raxibacumab does not significantly reduce immunogenicity of AVA. Serum anti-PA Abs measured in this study were host-derived anti-PA Abs elicited in response to vaccination with AVA. A validated immunodepletion method ensured no residual raxibacumab interfered with measurement of these Abs. Our results contradicted the earlier study in rabbits¹¹, this was despite our clinical study being designed to maximise the probability of raxibacumab and PA interacting; raxibacumab was infused immediately prior to AVA administration. Raxibacumab targets a well-conserved single epitope in domain IV of PA protein that binds to the host cell receptor. Several epitopes in PA are described to be associated with Abs with neutralising capability.²³ Thus, it is possible that as AVA contains PA from filtered bacterial culture supernatant, anti-PA Abs elicited during AVA vaccination may target these other epitopes in PA which are associated with neutralising Abs, even if raxibacumab is bound to its single epitope in PA, resulting in no significant immunological interference. Additionally, other minor constituents of AVA elicit an immune response which will not be inhibited by co-administration of raxibacumab. In contrast, polyclonal anthrax Ig administered to rabbits may form immune complexes with PA and other constituents of AVA, with binding at multiple sites potentially hindering immune recognition of multiple PA epitopes, leading to reduced AVA immunogenicity.¹¹

Six subjects (6/269, 2.2%) co-administered AVA and raxibacumab did not mount an anti-PA response at Weeks 4, 8, or 26. While it is not possible to exclude that this was due to interference between raxibacumab and AVA, this vaccine non-response rate was within the expected range for AVA. In a previous study, following SC administration of AVA, 19% of subjects at Week 4 and 5.1% at Week 8 (following an AVA dose at Week 4), had not responded with a ≥ 4 -fold rise in Ab titre.²⁴

TNA measures the ability of anti-PA Abs to protect living cells from a lethal dose of activated anthrax toxin; there is good correlation between anti-PA Ab and TNA titres.^{25,26} Seroconversion was higher at Week 4 in the AVA plus raxibacumab (99.3%) group compared with the AVA group (76.4%) in our study. This is as expected since the TNA assay does not differentiate between host-derived versus raxibacumab-associated serum neutralising activity. As raxibacumab concentration decreases at later time points, its impact on TNA also decreases and seroconversion rates were comparable at Weeks 8 and 26. However, the higher TNA GMT in the raxibacumab plus AVA group at Week 4 and 8 indicate additive and durable protection of raxibacumab over that produced by AVA alone.

There were no new significant safety findings in this study. Overall, the number of subjects who experienced AEs was similar in the groups, with no discernable significant differences in the safety findings in the AVA plus raxibacumab group compared with the known safety profiles of the individual products. Six subjects from the AVA plus raxibacumab group withdrew from the study due to infusion related reactions associated with administration of raxibacumab, which were considered mild/moderate and non-serious. None of these subjects were administered AVA. Monoclonal antibodies are known to be associated with infusion related reactions and hypersensitivity reactions (including anaphylaxis) and patients should be carefully monitored and treated appropriately should symptoms occur. Raxibacumab

systemic exposure levels in this study were comparable to those observed previously in healthy subjects,¹⁰ indicating that the presence of AVA does not interfere with serum raxibacumab disposition.

This study has implications for PEP recommendations for inhalational anthrax since the data provides support for co-administration of AVA with raxibacumab. Antibiotics administered following exposure to *B. anthracis* have been shown in animal models to be protective in the acute setting.⁷ Following the 2001 USA postal event, over 10,000 people were recommended to complete the full course of antibiotic prophylaxis, with no resultant case of disease. However, compliance rates to the full 60-day course recommended at the time were reported to be less than 50%.²⁷ Thus, the current prolonged course of antibiotics recommended for PEP is challenging, especially in the context of poor compliance in asymptomatic individuals. The recommended duration of PEP antibiotics was updated by the CDC in 2019; in immunocompetent healthy adults (18–65 years), antibiotics should be administered for 42 days after the first dose of anthrax vaccine or 2 weeks after the last dose of the vaccine series, whichever comes later. Individuals who are immunocompromised or in whom data on immune response to AVA is lacking (e.g. children, pregnant women, and adults ≥ 65 years) should continue to receive the previously recommended 60-day course.⁶ However, it is not anticipated that the compliance with the reduced 42-day course will significantly improve. Additionally, in certain circumstances antibiotics may be contraindicated for some patients, such as those with allergies, or infection may be caused by antibiotic-resistant strains. Thus, administration of a single dose of raxibacumab in addition to AVA may help to address these challenges. Furthermore, this study has potential wider implications in the context of increasing antimicrobial drug resistance. Monoclonal Abs are being evaluated for management of infectious diseases^{28,29} and vaccines are being developed, which may target the same components as the mAb.³⁰ Co-administration of vaccines with mAbs may result in

immunological interference, however, as demonstrated by our study, this may be reliably evaluated in controlled clinical trials.

In conclusion, these data support that co-administration of raxibacumab with SC AVA as part of a PEP regimen does not negatively impact the immunogenicity of AVA or the PK properties of raxibacumab. Moreover, the addition of raxibacumab to AVA in the setting of PEP has the potential to enhance disease prevention especially if antibiotics are ineffective or contraindicated during the early window when immunologic protection with vaccine is potentially insufficient.

Contributors

NS, VC, ODP, AY, JB, HS and LKT were involved in the study design and data analysis; WS contributed to the acquisition of data and data analysis; JW-J contributed to data analysis. JW-J and LKT drafted the manuscript and all authors were involved in the revision of the manuscript and approved the final version. Editorial support in the form of language editing and redrawing figures was provided by Lisa Auker, PhD, of Fishawack Indicia Ltd, UK (funded by GSK).

Declaration of interests

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Data sharing

Anonymised individual participant data and study documents can be requested for further research from www.clinicalstudydatarequest.com.

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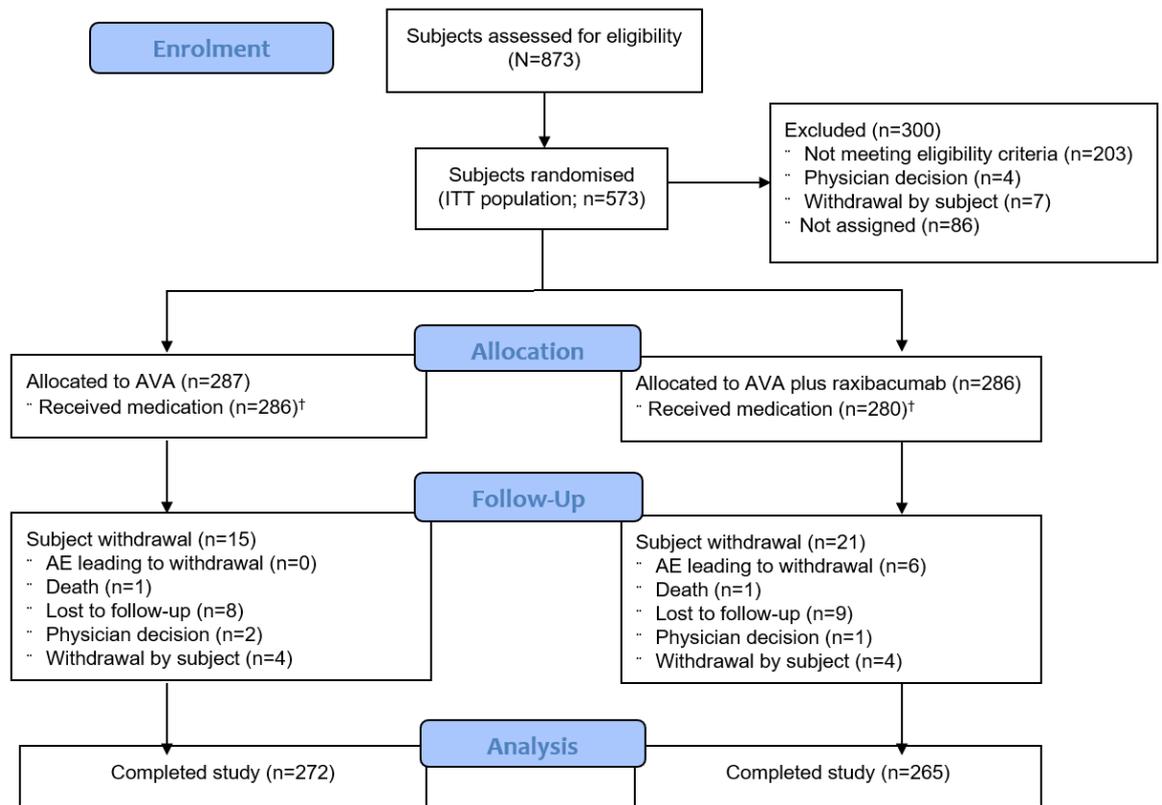
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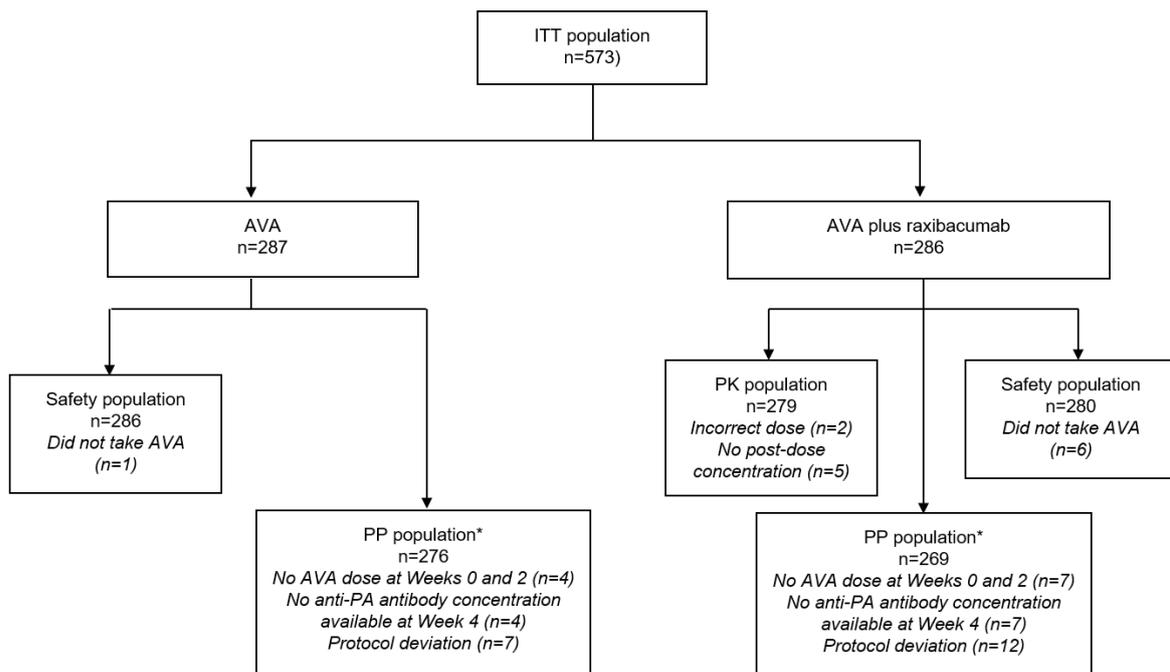
Figure legends

Figure 1. Subject disposition (CONSORT diagram) (A) and summary of study populations and exclusions (B).

A



B



AE, adverse event; AVA, anthrax vaccine adsorbed; ITT, intent-to-treat; PA, protective antigen; PK, pharmacokinetics; PP, per protocol.

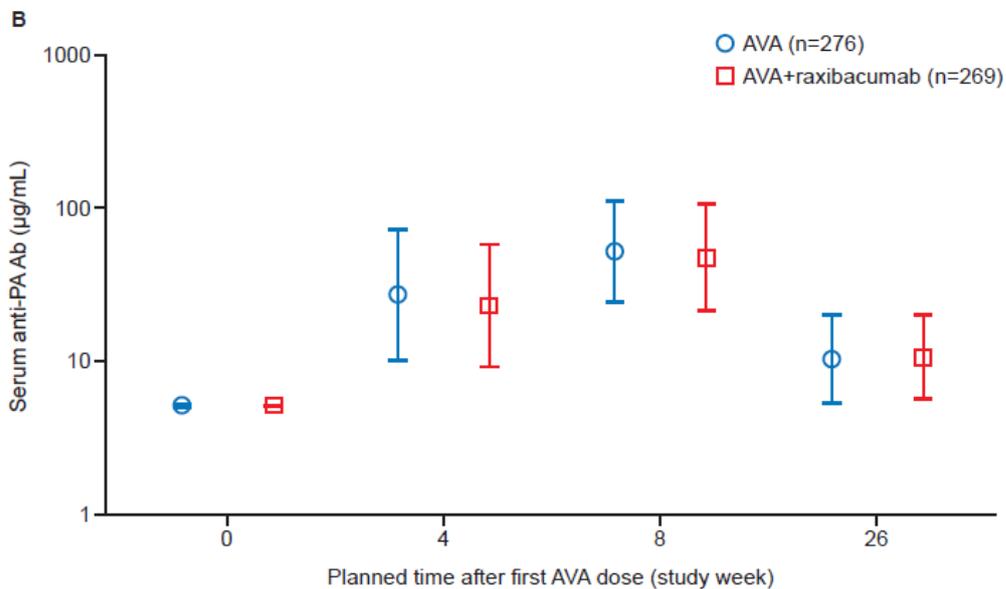
†One subject from the AVA-only group and six subjects from the AVA plus raxibacumab group did not receive the first AVA dose.

*The same subject may be excluded for more than one reason.

Figure 2. Comparison of geometric mean concentrations (GMCs) for anti-PA Ab (per protocol)

A

Week	AVA	AVA plus raxibacumab	Ratio of geometric means	90% CI	p value ¹
	n	n			
4	276 Geometric mean concentration, $\mu\text{g/mL}$ (95%CI) 26.5 (23.6, 29.8)	269 Geometric mean concentration, $\mu\text{g/mL}$ (95%CI) 22.5 (20.1, 25.1)	1.18	(1.03, 1.35)	0.0019
8	275 50.5 (46.1, 55.2)	267 46.1 (41.9, 50.8)	1.09	(0.98, 1.22)	<0.0001
26	267 10.0 (9.2, 10.8)	258 10.2 (9.5, 11.0)	0.98	(0.89, 1.07)	<0.0001



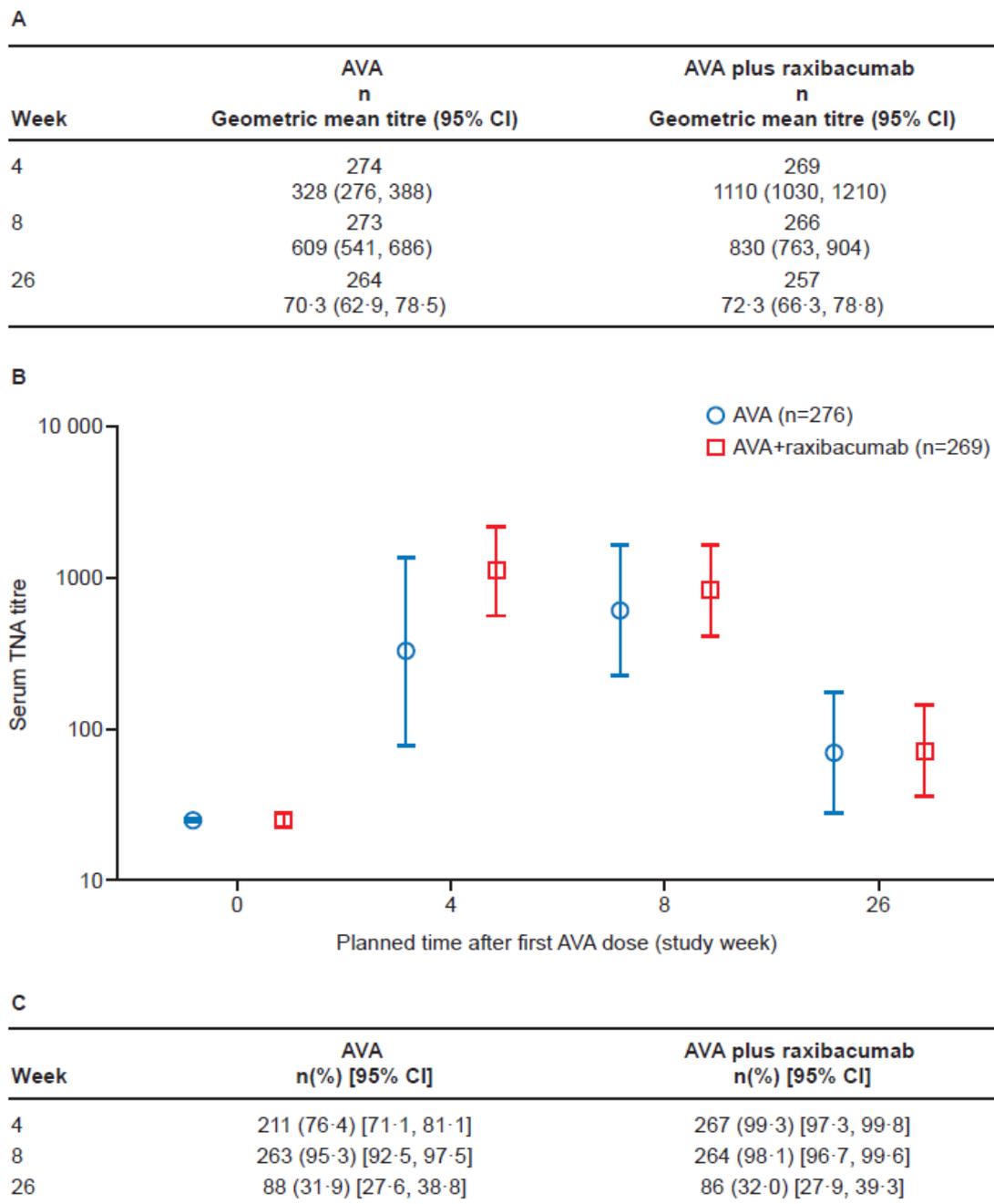
Panel A shows summary of statistical analysis of anti-PA Ab GMCs ($\mu\text{g/mL}$) at Week 4 (primary endpoint), Week 8 and 26 in the PP population.

Panel B shows geometric mean ($\pm\text{SD}$) plots representing anti-PA Ab concentration versus time of the AVA (blue circles) and AVA plus raxibacumab (red squares) groups in the PP population. Graph is plotted on a semi-logarithmic scale; error bars represent geometric SD.

¹p-value results are based on a one-sided, two-sample *t*-test for the null hypothesis that AVA/AVA plus raxibacumab ≥ 1.5 versus alternative hypothesis that AVA/AVA plus raxibacumab < 1.5 . This hypothesis was powered only for the primary analysis at Week 4.

Ab, antibody; AVA, anthrax vaccine adsorbed; CI, confidence interval; PA, protective antigen; PP, per protocol; SD, standard deviation.

Figure 3. Comparison of toxin neutralising activity (per protocol)



Panel A shows geometric mean titres (GMTs) of TNA, with corresponding 95% CIs, at Weeks 4, 8, and 26 in the AVA and AVA plus raxibacumab groups in the per protocol population.

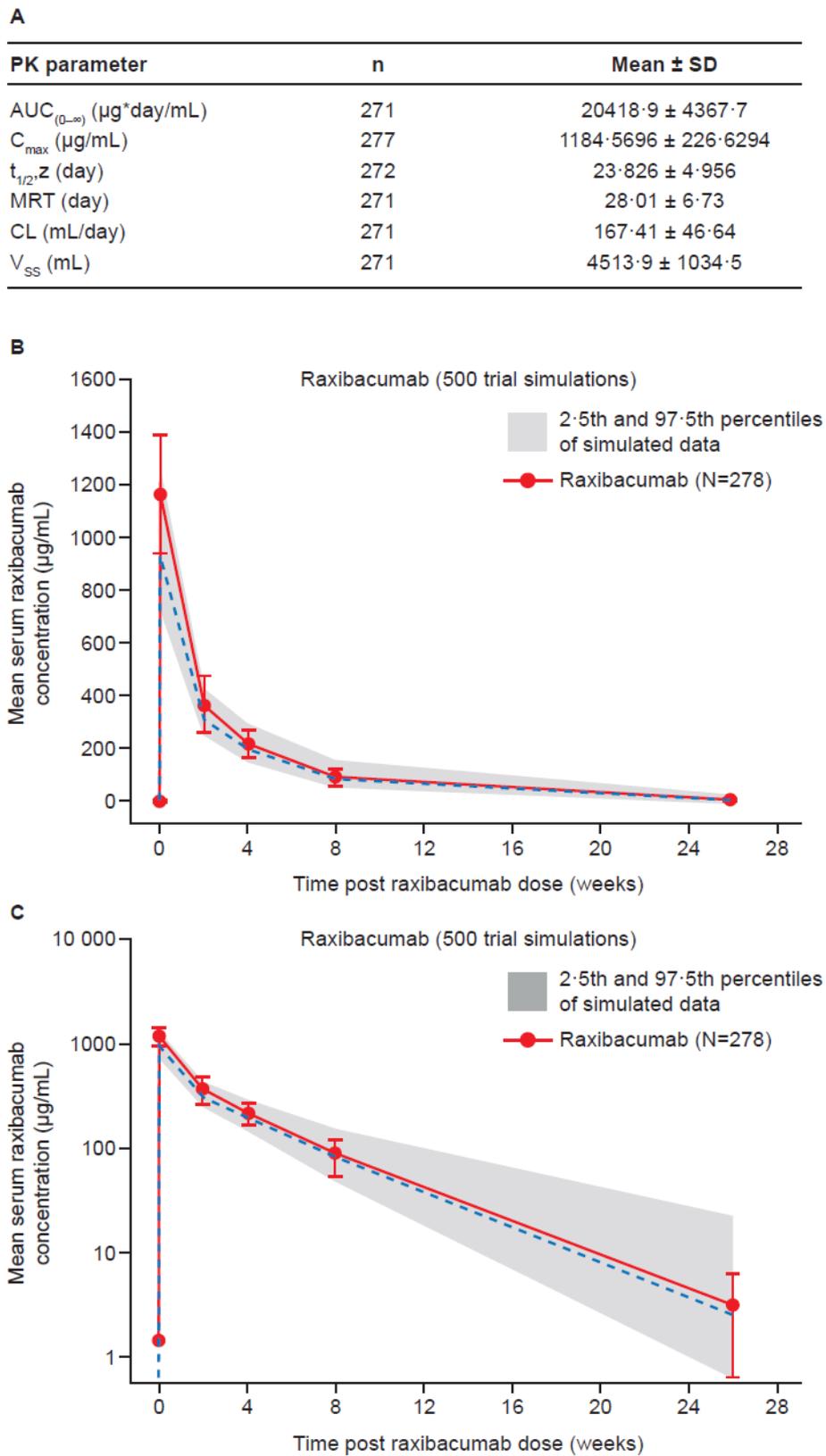
Panel B shows TNA titres at baseline and post-vaccination in the AVA (blue circles) and AVA plus raxibacumab (red squares) groups. Graph is plotted on a semi-logarithmic scale; error bars represent geometric SD.

Panel C shows percentage of subjects, with corresponding 95% CI, who achieved seroconversion at Weeks 4, 8, and 26 from the AVA and AVA plus raxibacumab groups. Seroconversion is defined as a ≥ 4 -fold increase in TNA titre from baseline; CIs for

seroconversion are based on Wilson's method. n (%): number (percentage) of subjects who seroconverted. Values below the LLoQ (25·2) were set to the LLoQ for calculation of summary statistics.

AVA, anthrax vaccine adsorbed; CI, confidence interval; PP, per protocol; TNA, toxin neutralising activity.

Figure 4. PK of raxibacumab in the AVA plus raxibacumab group



Panel A shows selected PK parameters from the PK population. AUC_(0-∞), area under the serum concentration-time curve from time zero to infinity; C_{max}, maximum serum

concentration; CL, clearance; MRT, mean residence time; PK, pharmacokinetics; SD, standard deviation; $t_{1/2,z}$, half-life of drug elimination in the terminal phase; V_{ss} , volume of distribution at steady-state.

Panel B shows a comparison of raxibacumab exposure between this study and previous clinical studies and population PK analyses on a linear scale. Panel C shows this comparison on a semi-logarithmic scale. Solid red line represents the mean observed concentration (+/-) SD. Whilst the PK population included 279 subjects, not all subjects had PK data collected at each sampling time. The PK profiles relate to a population of 278, i.e. the maximum number of subjects contributing to a given time point. Dashed blue and shaded grey area depict the predicted median profile and 95% confidence interval (n=500 simulations), respectively, based on the population PK of raxibacumab following administration of a 40 mg/kg dose in healthy subjects.