Statistical Analysis Plan

Title:	**A Randomized, Open-label Study to Evaluate the Immunogenicity of Anthrax Vaccine Adsorbed Alone or Concomitantly with Raxibacumab (GSK3068483)**
Protocol Number:	**201436**
Compound Number:	**GSK3068483**
Sponsor:	**GlaxoSmithKline Group of Companies** **1250 S. Collegeville Road** **Collegeville** **Pennsylvania, 19426**
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SIGNATURE PAGE

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Protocol: 201436

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Ab	Antibody
AE	Adverse Event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATC	Anatomic- therapeutic-chemical classification
$\%AUC_{ex}$	Percentage of $AUC_{(0-\infty)}$ obtained by extrapolation
AUC	Area under the curve
AUC_{0-t}	AUC from time zero to the time of last quantifiable concentration
$AUC_{0-\infty}$	AUC from time zero extrapolated to infinity
AVA	Anthrax vaccine adsorbed
BMI	Body mass index
BLQ	Below the limit of quantitation
C_{max}	Observed maximum concentration in the sampled matrix
CI	Confidence interval
CL	Clearance
CPMS	Clinical Pharmacology Modeling and Simulation
CV%	Coefficient of variation
eCRF	Electronic case report form
DMID	Division of Microbiology and Infectious Diseases
DMPK	Drug Metabolism and Pharmacokinetics
GMC	Geometric mean concentration
GMT	Geometric mean titer
GSK	GlaxoSmithKline
INR	International normalized ratio
ITT	Intent-to-treat
IV	Intravenous
λ_z	Apparent terminal rate constant
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LLOQ	Lower limit of quantitation
LS	Least-squares

MedDRA	Medical Dictionary for Regulatory Activities
MRT	Mean residence time
N	Population size (N for sample size, n for available data)
PA	Protective antigen
PDMP	Protocol deviation management plan
PK	Pharmacokinetics
PP	Per protocol
SAE	Serious adverse event
SAP	Statistical analysis plan
SC	Subcutaneous
SD	Standard deviation
SI	Système International
SOP	Standard Operating Procedures
$t_{1/2,z}$	Apparent terminal half-life
t_{max}	Time of maximum concentration (C_{max})
TEAE	Treatment-emergent adverse event
TNA	Toxin neutralizing activity
V_{ss}	Volume of distribution at steady-state
V_z	Volume of distribution in the terminal phase
UHPLC/MS	Ultra high performance liquid chromatography – mass spectrometry
ULN	Upper limit of normal

1.0 INTRODUCTION

The analyses outlined in this document are designed in support of clinical protocol 201436 dated 21 October, 2014. [1] This Statistical Analysis Plan (SAP) has been developed prior to database lock and interim or final analysis. Any deviations from the planned analyses described in this SAP will be documented in the clinical study report, together with the reason for such changes.

All final analyses will be conducted after the clinical trial data are entered into the database, any discrepancies in the data have been resolved, and the database is authorized (closed to changes). Prior to the start of any statistical analyses, the database must be authorized and all decisions regarding assignment of subjects to study populations must be completed. In addition, protocol deviations must be identified prior to the start of statistical analyses.

Pharmacokinetic (PK) analysis will be the responsibility of the pharmacokineticist at Quintiles. Analysis of safety data and immunogenicity of Anthrax Vaccine Adsorbed (AVA) data will be the responsibility of biostatistician at Quintiles.

Two interim analyses are scheduled during this study to determine if the futility threshold has been crossed at the respective analyses and whether the study should be continued. These analyses will be the responsibility of Quintiles.

2.0 STUDY DESCRIPTION

2.1 Study Objective(s)

2.1.1 Primary Objective(s)

- To compare the immunogenicity of AVA at 4 weeks after the first AVA dose, when AVA is administered alone or concomitantly with raxibacumab.

2.1.2 Secondary Objective(s)

- To compare the immunogenicity of AVA at Week 8 and 26 after the first AVA dose, when AVA is administered alone or concomitantly with raxibacumab.

- To estimate and compare seroconversion, defined as a $\geq$ 4-fold increase in toxin neutralizing activity (TNA) titer from baseline, at Weeks 4, 8 and 26 after the first AVA dose, when AVA is administered alone or concomitantly with raxibacumab.

- To describe the safety of AVA when co-administered with raxibacumab versus AVA alone.

2.2 Study Endpoint(s)

2.2.1 Primary Endpoint(s)

- Ratio of geometric mean concentrations (GMC) of anti-protective antigen (PA) antibody (Ab) at 4 weeks after the first AVA dose (prior to the third AVA dose), between the AVA alone and the AVA with raxibacumab treatment groups.

2.2.2 Secondary Endpoint(s)

- Ratio of GMC of anti-PA Ab at Weeks 8 and 26 after the first AVA dose, between the AVA alone and the AVA with raxibacumab treatment groups.

- Percentage of subjects who seroconvert, at Weeks 4, 8, and 26 after the first AVA dose, between the AVA alone and the AVA with raxibacumab treatment groups.

- Rates of reported adverse events (AE) as well as review of vital signs, urinalysis, and clinical laboratory data in the AVA alone and the AVA with raxibacumab treatment groups.

2.3 Overall Study Design and Plan

This is a Phase 4, randomized, open-label, parallel group, two arm study in healthy subjects who have not previously been immunized against the PA of *Bacillus anthracis*. Although this is an open-label study, measures have been introduced to minimize bias.

Subjects will be randomized into one of the following two treatment groups:

- Treatment Group 1 – subjects will be administered subcutaneous (SC) 0.5 ml AVA doses on Days 1, 15, and 29 (0, 2, and 4 weeks),

- Treatment Group 2 – subjects will be administered SC 0.5 ml AVA doses on Days 1, 15, and 29 (0, 2, and 4 weeks), with the first AVA dose administered immediately after completion of a single 40 mg/kg intravenous (IV) infusion of raxibacumab. Subjects will be premedicated with diphenhydramine (25-50 mg) up to 1 hour prior to the raxibacumab infusion to reduce the risk of infusion reactions.

Subjects will begin the study with a screening visit up to 35 days before the start of study treatment on Day 1. Results of the screening test for the anti-PA Ab levels must be available prior to randomization on Day -1. Study visits will occur at Days -1, 1, 15, 29, 57, and 183

(Week 26). Beginning with the screening visit, the total duration of study for each subject may be approximately 28 to 31 weeks.

A total of at least 534 subjects (267 analyzable subjects per treatment group) may be enrolled in the study, in 3 cohorts separated by interim analyses. The trial will continue to subsequent cohorts only if the prospective criteria for discontinuing the trial are not met at the interim analysis. If the outcome of this trial shows a large impact on immunogenicity, then the trial will likely be stopped at one of the two interim analyses.

Subjects will be enrolled in the study in up to 3 cohorts separated by 2 interim analyses. The trial will continue to subsequent cohorts only if the prospective criteria for discontinuing the trial are not met at the interim analysis.

- at least 30 subjects will be enrolled in Cohort 1,

- at least an additional 70 subjects will be enrolled in Cohort 2 if the study progresses beyond interim 1.

- and at least an additional 434 subjects will be enrolled in Cohort 3 if the study progresses beyond interim 2.

If a subject prematurely discontinues the study prior to the Day 29 primary endpoint measurement, additional subjects will be randomized to reach the required number of analyzable subjects for a given analysis (please refer to Section 4.3.3 for definition of an analyzable subject). The goal will be to only enroll the minimum number of subjects to reach the target number of analyzable subjects. Although the number of randomized subjects who will not be analyzable is unknown, based on expectations of study withdrawal and data from previous trials with AVA, it is estimated that no more than 620 subjects will be randomized to reach the target of 534 analyzable subjects. The decision to randomize additional subjects will be made without knowledge of the ongoing comparative results; if ongoing comparative results for the current cohort are known to the Sponsor, no additional subjects may be enrolled.

2.4   Sample Size

The sample size calculation is based on the assumption that the individual values will be analyzed after being transformed by $\log_{10}$ resulting in data that is approximately normal. In the transformed data the analysis of the ratio of means is equivalent to a difference of mean values with a margin of $\log_{10}(1.5) = 0.176$. The standard deviation of the $\log_{10}$ transformed data is assumed to be 0.75.

The sample size is calculated assuming 1:1 randomization and two interim analyses for futility after 30 and 100 analyzable subjects. The boundaries for the futility analysis are based on Rho family error spending function with a rho of 0.5. For the purposes of the sample size calculation, the power will be calculated under the alternative hypothesis setting, where the ratio is 1, or equivalence between the two arms with respect to concentrations of anti-AP antibody. With a one-sided type I error of 5% a sample size of 534 subjects will allow for 80% power to make a claim of non-inferiority (margin of 1.5), if the true ratio between the geometric means is 1.

In a trial of 534 patients, the largest observed GMC ratio between the AVA and the combination that results in a statistically significant non-inferiority result is an estimated ratio of 1.173. This can alternatively be viewed as a minimum observed anti-PA concentration due to the addition of AVA in the combination of ~85% of the concentration when AVA is administered alone. This is under the assumption that the observed variability in the study population is similar to the study design assumption.

Details on the sensitivity analyses of sample size determination can be found in Section 9.2.2 of the study protocol.

2.5   Method of Assigning Subjects to Treatment Groups

Subjects will be assigned to Treatment Group 1 or Treatment Group 2 in accordance with a balanced independent randomization schedule generated prior to the start of the study. A corresponding set of randomization envelopes (one per subject) will be generated. A randomization envelope for each subject will be opened just prior to dosing to reveal the subject's treatment assignment. This process will ensure that study personnel and the volunteers will have no knowledge of the next treatment to be assigned.

Once a randomization number has been assigned to a subject, it will not be re-assigned to another subject. Randomization numbers will be assigned sequentially as subjects become eligible for dosing.

To reduce the delay associated with randomizing additional subjects, 34 subjects will be randomized to achieve at least 30 analyzable subjects for the first interim analysis in Cohort 1.

2.6   Study Schedule

The study will be comprised of a screening period (Days -36 to -15) and a treatment period (Days -1 to 183 ± 4). The complete schedule of visits and all procedures is presented in Table 2. 1, Table 2. 2, and Table 2. 3

Table 2. 1   Screening assessments for subjects administered AVA or co-administered raxibacumab and AVA

Screening Assessments	Days -36 to -15[a]	Notes
Informed consent process	X	
Demographic information	X	
Inclusion/Exclusion criteria	X	
Medical history (includes substance usage and Family history of premature CV disease)	X	
Physical exam including height and weight	X	
Alcohol and Drug Screening	X	
Urinalysis	X	
Vital signs	X	
Hematology and clinical chemistry	X	Refer to Table 4 in CSP [1] for specific laboratory tests.
HIV, Hep B and Hep C screen	X	
Review concomitant medications	X	
Serum or urine pregnancy test	X	
AE/SAE assessment	X	Will capture SAEs reported between Screening visit and Day -1.
Toxin Neutralizing Ab (TNA)	X	
Anti-PA Ab	X	

a. The screening window closes at Day -15 to allow all analyses to be completed to confirm subject eligibility.

Table 2. 2   Treatment Group 1: Time and Events for subjects administered AVA

Protocol Activity	Study Visit						Notes
Assessments	Day -1[a]	Day 1	Day 15 (± 2 days)	Day 29 (± 2 days)	Day 57 (± 2 days)	Day 183 (± 4 days)	
Inpatient visit	←X→						
Outpatient visit			←-------X-------→				
Urinalysis					X		
Admit to unit	X						
Randomization	X						
Discharge from unit		X					The subject will be discharged from the unit after all assessments are completed.
Vital signs	X	X		X	X	X	
Hematology and clinical chemistry	X			X	X		Refer to Table 4 in CSP [1] for specific laboratory tests. On Day 29 the specimen will be collected
Review concomitant medications	X		X	X	X	X	
Serum or urine pregnancy test	X		X	X	X	X	
AVA administration		X	X	X			Each sequential AVA injection will be administered in a different injection site (e.g. alternating arms).
AE/SAE assessment	X	X	X	X	X	X	
Toxin Neutralizing Ab (TNA)		X		X	X	X	On Days 1 and 29 the specimen will be collected prior to AVA injection.
Anti-PA Ab		X		X	X	X	On Days 1 and 29 the specimen will be collected prior to AVA injection.

a.   Day -1 is the calendar day preceding Day 1; the designation "Day 0" is not used in this study.

Table 2. 3   Treatment Group 2: Time and Events Table for subjects co-administered raxibacumab and AVA

Protocol Activity	Study Visit									Notes
		Day 1								
Assessments	Day -1[a]	pre-raxi infusion	0 hr	0.5 hr (± 5 min)	2-6 hrs	Day 15 (± 2 days)	Day 29 (± 2 days)	Day 57 (± 2 days)	Day183 (± 4 days)	
Inpatient visit	←----------X----------→									
Outpatient visit						←----------X----------→				
Urinalysis								X		
Admit to unit	X									
Randomization	X									
Discharged from unit					X					The subject will be discharged from the unit after all assessments are completed.
Vital signs	X				X		X	X	X	
Hematology and clinical chemistry	X						X	X		Refer to Table 4 in CSP [1] for specific laboratory test. On Day 29 the specimen will be collected prior to treatment administration.
Review concomitant medications	X					X	X	X	X	
Serum or urine pregnancy test	X					X	X	X	X	
Diphenhydramine administration		X								Diphenhydramine (25-50 mg) must be administered within 60 minutes prior to the raxibacumab infusion.
Raxibacumab administration			X							Raxibacumab infusion must be completely administered prior to first AVA dose on Day 1 and administered in opposite limb from AVA injection.
AVA administration			X			X	X			Each sequential AVA injection will be administered in a different injection site (eg. alternating arms).
AE/SAE assessment	X	X		X	X	X	X	X	X	
PK sampling		X		X	X	X	X	X	X	A blood specimen will be collected prior to raxibacumab infusion, 30 minutes post raxibacumab infusion (± 5 min), 2-6 hours post infusion on Day 1, and at Days 15, 29, and 57 (14, 28, and 56 days post raxibacumab administration). An additional sample will be collected at the Day 183 visit.
Anti-raxibacumab Ab		X						X	X	
Toxin Neutralizing Ab (TNA)		X					X	X	X	The Day 29 specimens will be collected prior to AVA administration.
Anti-PA Ab		X					X	X	X	The Day 29 specimens will be collected prior to AVA administration.

a.   Day -1 is the calendar day preceding Day 1; the designation "Day 0" is not used in this study.

3.0   ANALYSIS VARIABLES

3.1   Pharmacokinetic Variables

Blood samples for pharmacokinetic (PK) analysis of raxibacumab will be collected from all the subjects in the AVA/raxibacumab treatment group at the time points indicated in Section 2.7, Study Schedule. The actual date and time of each blood sample collection will be recorded. The timing of PK samples may be altered and/or PK samples may be obtained at additional time points to ensure thorough PK monitoring.

Serum raxibacumab concentrations will be determined using an approved method based on immunocapture and trypsin digest, followed by UHPLC/MS/MS analysis, by Drug Metabolism and Pharmacokinetics (DMPK), GlaxoSmithKline (GSK).

3.2   PA, TNA and Anti-raxibacumab Antibodies

Serum anti-PA Ab concentrations will be determined using immunoassay by DMPK, GSK. This analysis will measure concentrations of a combination of raxibacumab as well as anti-PA antibodies due to AVA. Concentrations of anti-PA Ab due to vaccine will be the basis of the primary analysis. This will be calculated by subtracting serum raxibacumab concentrations from the anti-PA assay result for the corresponding collection time and subject, by GSK DMPK. Serum TNA titers will be determined using a cell-based assay, under the auspices of Clinical Immunology, GSK.

Serum anti-raxibacumab Ab analysis will be performed under the control of Clinical Immunology, GSK. The presence of anti-raxibacumab Ab will be determined using validated ECL bridging assay.

3.3   Safety Variables

The safety of AVA when co-administered with raxibacumab versus AVA alone will be assessed on the basis of:

- Adverse events (AEs) collected from the start of study treatment until the follow-up contact;

- Clinical laboratory evaluations; and

- Vital signs;

See Table 2. 1, Table 2. 2, and Table 2. 3 for the details of evaluation time points.

4.0 STATISTICAL METHODOLOGY

4.1 General Considerations

Analysis of the PK of raxibacumab, will be the responsibility of the pharmacokineticist at Quintiles, Overland Park, Kansas, United States. The PK and safety summaries and data listings as well as the statistical analysis of the immunogenicity data will be the responsibility of the unblinded (interim analyses) and blinded (final analysis) biostatistician or pharmacokineticist at Quintiles, Overland Park, according to Quintiles Standard Operating Procedures (SOPs).

For qualitative variables, the population size (N for sample size and n for available data) and the percentage (of available data) for each class of the variable will be presented. Quantitative variables will be summarized using descriptive statistics including N and/or n, mean, standard deviation (SD), median, minimum, and maximum values. Geometric mean and geometric CV%, defined as $100 \cdot \sqrt{\left(\exp\left(s^2\right) - 1\right)}$ where s is the SD of the data on a log scale, will be included for PK and immunogenicity parameters, where applicable.

Pharmacokinetic parameters will be rounded for reporting purposes both in the summary tables and by-subject listings. For the calculation of descriptive statistics and the statistical analysis, rounded values as presented in the data listings will be used. All data will be reported and analyzed with the same precision as the source data regardless of how many significant figures or decimals the data carry. For most PK parameters, 3 significant digits will be used as the standard rounding procedure, with the following exceptions:

- Parameters directly derived from source data (e.g. C_{max}) will be reported and analyzed with the same precision as the source data.

- Parameters derived from actual sample collection times (e.g. t_{max}) will be reported with the same precision as the actual sampling time value of the source data

Reporting of minimum and maximum will follow the rounding convention of the source variables. Reporting of mean, SD, median, and bounds of all confidence intervals (CI) will include an additional significant digit. Coefficient of variation (CV%) will always be reported to 1 decimal place.

All available data from subjects receiving at least 1 dose of AVA will be included in the safety analyses if valid. Extra measurements (such as unscheduled or repeat assessments) will not be included in the by-visit summary, but will be included in subject listings. All adverse events and clinical laboratory outliers that occur following the first dose of study medication will be included in the presentations of adverse events and outlier episodes, including episodes that occur at unscheduled evaluations.

Study disposition will be presented for All Subjects Enrolled population as defined in Section 4.2.1. Demographics will be presented for Intent-to-Treat population as defined in Section 4.2.2. Immunogenicity summaries will be presented for all subjects in the Per Protocol population as defined in Section 4.2.3. Safety summary statistics will be presented for all subjects in the Safety population as defined in Section 4.2.4. Data from subjects excluded from an analysis population will be included in the data listings, but not in the summaries.

Baseline, defined as the last scheduled observation prior to dosing, will correspond to Day -1 for clinical laboratory evaluations and vital signs and to Day 1, prior to administration of any study drug, for anti-PA Ab levels and TNA titers. Additionally, for subjects randomized to Treatment Group 2, baseline will correspond to Day 1, prior to administration of any study drug, for anti-raxibacumab Ab levels. However, if a subject is missing the baseline collection, the previous non-missing evaluation will become the baseline value. If no baseline or previous to baseline evaluations exist then the baseline value will be treated as missing.

In general, data will be presented by treatment (i.e., "AVA" and "Raxibacumab + AVA"). Data for all study subjects combined will also be presented when appropriate.

Some minor modifications may be necessary to the planned design of tables, figures, and listings to accommodate data collected during the actual study conduct.

4.2   Analysis Populations

4.2.1 All Subjects Enrolled Population

The All Subjects Enrolled population will include all subjects who consent to participate in the clinical trial. Subjects in this population will be used for reporting screen failures.

4.2.2 Intent-to-Treat Population

The Intent-to-Treat (ITT) population will comprise all randomized subjects. Subjects in this population will be used for sensitivity analyses.

4.2.3 Per Protocol Population

The Per Protocol (PP) population will comprise all analyzable subjects and will be used for the primary analysis. The PP population is a subset of ITT population. A subject will be considered analyzable if they meet all of the following criteria:

1. Receive at least the Week 0 (Day 1) and Week 2 (Day 15) AVA doses within the protocol specified visit window,

2. Receive the raxibacumab dose, if randomized to Treatment Group 2

3. Complete the primary study endpoint assessment (anti-PA Ab concentration at Week 4).

Subjects meeting the above criteria will be identified as such on the electronic case report form (eCRF). Appendix I of the protocol deviation management plan (PDMP) lists all the conditions related to eligibility, consent, wrong study treatment, administration, dose that may lead to exclusion of a subject from the PP population.

4.2.4 Safety Population

The Safety population will comprise all randomized subjects receiving at least one dose of AVA and will be based on the actual treatment received, particularly, if this differs from that to which the subjects were randomized. That is, subjects randomized to receive both raxibacumab and AVA but only receive AVA will be summarized with the other subjects who only received AVA. Subjects in this population will be used for all safety summaries. Subjects who only receive raxibacumab will not be included in the safety population summaries.

Note: The Intent-to-Treat population and Per Protocol population will be analyzed as-randomized, rather than as-treated, in contrast to the Safety population.

4.3 Incomplete and Missing Data

For safety summaries, no adjustment or imputation will be utilized for missing values or for subjects who withdraw prior to completing the study. Also, no analyses will be restricted to subjects with complete data.

4.4 Protocol Deviations

All protocol deviations will be tracked by the study team throughout the conduct of the study in accordance with the PDMP. See Appendix I of PDMP for a list of protocol deviations that are considered important. Data will be reviewed prior to unblinding and freezing the database to ensure all important deviations and deviations which may lead to exclusion from the analysis populations are captured and categorized on the protocol deviations dataset. This dataset will be the basis for the summaries and listings of protocol deviations, as well as the definition of the Per Protocol population.

- Important protocol deviations (including deviations related to study inclusion/exclusion criteria, conduct of the trial, patient management or patient assessment) will be summarized by category and listed.

- Important deviations which result in exclusion from the analysis population will also be summarized by category and listed.

4.5   Subject Disposition

A subject will be considered enrolled at the time of providing informed consent. Subjects will be considered to have completed the study if they have completed all study visits including the follow-up visit.

Subject accountability will be tabulated by treatment and for all subjects overall by summarizing the number of subjects who enrolled (i.e., provide the informed consent), are randomly assigned to treatment, complete the study, or prematurely discontinue along with the reason for early discontinuation. A listing will be presented to describe dates of completion or early withdrawal and the reason for early discontinuation, if applicable, for each subject.

Listings of study eligibility, treatment randomization, and study treatment administration will be provided.

4.6   Demographic and Baseline Characteristics

Individual subject demographics and baseline characteristics (medical history and results from urine drug and alcohol screens, serology screening, and pregnancy tests) will be presented in listings.

Demographic characteristics such as age, sex, race, ethnicity, height, weight, and body mass index (BMI) will be summarized and tabulated by treatment and for all subjects overall. Descriptive statistics will be presented for age, height, weight, and BMI. Frequency counts and percents will be presented for sex, race, and ethnicity.

4.7   Previous and Concomitant Medications

Previous medications are defined as any medication discontinued prior to the first dose of study drug. Concomitant medications are defined as any medication taken during the course of the study. A subject listing and a summary of all previous and concomitant medications will be presented. All medications will be coded and classified by anatomic-therapeutic-chemical classification (ATC) Level 1 Code using the GSK Drug System.

See Appendix A for handling of partial dates for medications. In the case where it is not possible to define a medication as prior, concomitant, or post-treatment, the medication will be classified as concomitant.

4.8   Pharmacokinetic Analyses

A listing of PK blood sample collection times as well as derived sampling time deviations will be provided.

Drug concentration-time data will be listed for each subject in Treatment Group 2 and summarized by descriptive statistics at each nominal collection time. Below the limit of quantitation (BLQ) concentrations will be treated as zero for computation of descriptive statistics. Concentrations assigned a value of missing will be omitted from the calculation of descriptive statistics. If the calculated mean concentration is BLQ, the mean value will be reported as BLQ. SD will be reported as ND (not determined). Minimum, median, and maximum may be reported. If any of these values are BLQ, they will be reported as such and indicated as BLQ.

Figures of mean raxibacumab concentration-time data in Treatment Group 2 will be presented on linear and semi-logarithmic scales. Individual subject concentration-time data will be graphically presented on linear and semi-logarithmic scales.

Predose samples that are BLQ or missing will be assigned a numerical value of zero for the calculation of area under the concentration-time curve (AUC). Predose samples that are missing will be excluded from the concentration summary calculations. Any anomalous concentration values observed at predose will be identified in the study report and used for the computation of AUC. Pharmacokinetic parameters will be computed and reported if the anomalous value is not greater than 5% of the observed maximum concentration (C_{max}). If the anomalous value is greater than 5% of C_{max}, the PK parameters for the given subject will be computed in the PK analysis but will not be reported.

Any other BLQ concentrations that precede quantifiable samples in the initial portion of the profile or a BLQ value that occurs between quantifiable data points, especially prior to C_{max}, will be evaluated to determine if an assigned concentration of zero makes sense, or if reanalysis or exclusion of the data is warranted. Following C_{max}, BLQ values embedded between 2 quantifiable data points will be treated as missing when calculating AUC. If BLQ values occur at the end of the collection interval (after the last quantifiable concentration), these will be treated as missing data. If consecutive BLQ concentrations are followed by quantifiable concentrations in the terminal portion of the concentration curve, these quantified values will be excluded from the PK analysis by assigning them a value of missing, unless otherwise warranted by the concentration-time profile.

Specimens will also be analyzed for the presence of anti-raxibacumab antibodies. Those results will be descriptively and/or graphically summarized as appropriate to the data.

The following plasma PK parameters will be estimated by noncompartmental methods for serum raxibacumab if data permits:

C_{max}	Maximum observed serum raxibacumab concentration.
t_{max}	Time of occurrence for C_{max}.
AUC_{0-t}	Area under the serum raxibacumab concentration-time curve to the time of the last measurable concentration.
$AUC_{0-\infty}$	Area under the serum raxibacumab concentration-time curve to infinite time.
λ_z	Terminal elimination rate constant.
$t_{1/2,z}$	Half-life of drug elimination in the terminal phase.
MRT	Mean residence time.
CL	Clearance.
V_z	Volume of distribution in the terminal phase.
V_{ss}	Volume of distribution at steady-state.

The following PK parameters will be calculated for diagnostic purposes and listed, but will not be summarized:

$t_{1/2}$,Interval	The time interval (days) of the log-linear regression to determine $t_{1/2}$
$t_{1/2}$,N	Number of data points included in the log-linear regression analysis
Rsq	Goodness of fit statistic for calculation of λ_z (Regression coefficient). If Rsq is less than 0.800, λ_z and related parameters will not be reported.
%AUC_{ex}	Percentage of $AUC_{0-\infty}$ obtained by extrapolation, calculated as $[(C_{last}/\lambda_z)/AUC_{0-\infty} *100]$

A subject listing of individual PK parameters will be provided. Pharmacokinetic parameters will be summarized using descriptive statistics. For t_{max} only mean, minimum, maximum and median will be reported.

4.9 Interim Analyses

Two interim analyses are scheduled to allow early termination of the study if a large effect of raxibacumab on AVA immunogenicity is confirmed at the interim. The first interim analysis will be performed after at least 30 analyzable subjects have reached the Week 4 endpoint. The trial is randomizing 34 patients to ensure that at least 30 subjects are analyzable, so it is possible that there will be more than 30 patients analyzed in the first interim. The second interim will be performed after the first 100 analyzable subjects have reached the Week 4 endpoint. There may also be additional subjects randomized to meet the total of 100 analyzable subjects for the second interim. The final number enrolled will be informed by the level of drop-outs observed in the first 34 subjects randomized.

These interim analyses will be performed on the per-protocol population by independent unblinded statistical and medical personnel using unblinded data.

For the interim analyses, the immunogenicity data will be summarized by the ratio of geometric mean concentrations (GMC) of anti-PA antibody levels with corresponding 95% CI. A two sample t-test for the log transformed data will be performed and a corresponding p-value will be provided which will be used to determine if the futility threshold has been crossed at the respective interim analyses. No imputations will be utilized for interim analyses. The study will be terminated early if the futility boundary is crossed at an interim, otherwise, recruitment for next phase will begin.

The futility thresholds for the interim analyses will be based on a rho (0.5) type II error spending function. The t-statistics for the boundaries, as calculated in East 5.4, are 1.0274 for interim 1 (sample size = 30 subjects), 0.4437 for interim 2 (sample size = 100 subjects) and -1.645 for the final analysis. These correspond to p-values for the t-test of p=0.848 at interim 1, p=0.671 at interim 2, and p=0.05 for the final analysis.

Note: The futility boundary will be recalculated by the unblinded statistician based on the actual number of subjects who comprise the primary analysis population for cohort 1. These boundaries will be calculated using East 6.2.

For these interim analyses, the results will include:

- calculations of futility boundaries (Table 14.2.3.1.1);

- a summary of statistical comparison of anti-PA antibody levels (Table 14.2.3.1.2);

- a demographic summary (Table 14.1.2);

- a listing and a summary of the observed AEs (Listing 16.2.7.2, Table 14.3.1.2);

- a listing of serious AEs (Listing 14.3.2.2);

- a listing of AEs leading to withdrawal from study (Listing 14.3.2.3);

- a listing and a summary of laboratory assessments (Listing 16.2.8.1.1, Table 14.3.6.1, Table 14.3.6.2); and

- a listing and a summary of vital signs (Listing 16.2.8.2.1, Table 14.3.6.5, Table 14.3.6.6).

4.10   Statistical Hypotheses

The null hypothesis is that the ratio of the GMC of anti-PA Ab attributable to AVA in the AVA arm to the GMC of anti-PA Ab in the AVA+Raxibacumab arm is greater than or equal to 1.5 (non-inferiority margin). The alternative hypothesis is that the ratio is less than 1.5.

The null and alternative hypotheses are as follows:

H_0: $\theta = \mu AVA / \mu AVA+Raxibacumab \geq 1.5$

H_A: $\theta = \mu AVA / \mu AVA+Raxibacumab < 1.5$

Where θ is the ratio of GMCs; μAVA and $\mu AVA+Raxibacumab$ are the population GMCs of the anti-PA Ab attributable to AVA for the two arms.

4.11   Primary Analysis

At the final analysis, the non-inferiority test will be based on the CI for the ratio of anti-PA Ab (attributable to AVA) GMCs between the AVA and the AVA + raxibacumab groups at Week 4. This analysis will be performed in the per-protocol population. If the upper bound of the 90% CI is less than 1.5, non-inferiority will be established.

This analysis will be repeated in a sensitivity analysis which will use the full ITT population with imputation for subjects without Week 4 anti-PA Ab concentration results. Two methods of imputation will be used for subjects without Week 4 anti-PA Ab concentration results:

1. In the AVA + raxibacumab arm, missing data will be imputed with the first quartile from the non-missing data in this arm. In the AVA arm, missing data will be imputed with the third quartile from the non-missing data in this arm.

2. In the AVA + raxibacumab arm, missing data will be imputed with the lower limit of quantitation (LLQ). In the AVA arm, missing data will be imputed with the maximum from the non-missing data.

The GMCs for each treatment group will be summarized with corresponding 95% CI for the Week 4 timepoint as well as Week 8 and Week 26.

The GMC ratio and GMCs within each cohort and corresponding CIs will be calculated by first log transforming all of the data by a log base 10. A t-test will be performed and CIs will be calculated about the mean logs and the difference of the mean logs. The results will be transformed back to the original scale by exponentiation, again using a base 10. The exponentiated differences of the sample mean logs will be the ratio of the sample geometric means of the untransformed results.

Welch's unequal variances *t*-test will be performed to assess the sensitivity of the results to the assumption of equality of variances between the anti-PA Ab concentrations in the two treatment groups.

4.12   Secondary Analysis

4.12.1 Anti-PA Antibodies

Anti-PA antibody concentrations will also be collected at Weeks 8 and Week 26 after the first AVA dose. Values less than BLQ will be set to lower limit of quantitation (LLOQ). The GMCs with corresponding 95% CI will be summarized for each treatment group at each timepoint. No comparison between treatments will be made. There will be no imputation for subjects with missing data at a given timepoint.

4.12.2 Toxin Neutralizing Activity (TNA)

Toxin neutralizing activity titers will be collected at weeks 4, 8 and 26 after the first AVA dose. The geometric mean titers (GMT) with corresponding 95% CI will be summarized for each treatment group at each timepoint. There will be no imputation for subjects with missing data at a given timepoint.

The percentage of subjects, with corresponding 95% CI, who seroconvert, defined as a $\geq$ 4-fold increase in TNA titer will be summarized, at Weeks 4, 8, and 26 after the first AVA dose for both arms separately.

4.12.3 Statistical Analysis of Pharmacokinetic Data

Drug concentration-time data will be listed for each subject and summarized by descriptive statistics at each nominal collection time. Pharmacokinetic parameters will be listed for each subject and summarized by descriptive statistics. Specimens will also be analyzed for the presence of anti-raxibacumab antibodies. Those results will be descriptively and/or graphically summarized as appropriate to the data.

4.12.4 Safety Analyses

All safety assessments, including AEs, clinical laboratory evaluations, and vital signs will be listed and where appropriate summarized with descriptive statistics.

4.12.4.1 Summary of Exposure

Study treatment administration will be listed and summarized for AVA with the number (%) of subjects receiving dose on Day 1, Day 15 and Day 29.

4.12.4.2 Adverse Events

An AE is any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. The period of observation for AE reporting will begin from the start of study treatment until the follow-up contact.

All AEs will be coded to body system and preferred term using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary, 17.1.

A treatment-emergent adverse event (TEAE) is defined as any AE that began or worsened following the start of dosing. A treatment-related AE is defined as any TEAE that is assessed by the Investigator as having a reasonable possibility that the AE may have been caused by the study treatment or those for which the relationship is missing.

The total number and percentage of subjects with TEAEs will be presented by system organ class and preferred term for each study treatment and for all subjects overall. Treatment-emergent adverse events will also be presented by maximum grade according to the Division of Microbiology and Infectious Diseases (DMID) toxicity tables (Appendix 6 of the study protocol). For AEs not included in the appendix above, the following guidelines will be used:

Mild: An event causing no limitation of usual activities.

Moderate: An event causing some limitation of usual activities.

Severe: An event causing inability to carry out usual activities.

Life threatening: An event that is potentially life threatening or disabling.

Treatment-emergent adverse events will be tabulated presenting the system organ class alphabetically and within each system the preferred term will be presented in decreasing order of the total number of subjects who experienced each TEAE. Treatment-related adverse events will be similarly presented.

To count the number of subjects who experience each TEAE or treatment-related AE, a subject experiencing the same AE multiple times will only be counted once for that preferred term. Similarly, if a subject experiences multiple AEs within the same system organ class, that subject will be counted only once for that system. If changes in the DMID grade/severity of an AE are recorded in the eCRF, only the maximum grade/most severe incidence of the AE will be counted. For the summary of treatment-related AEs, if a subject experiences multiple occurrences of a TEAE then the subject will be counted as having a treatment-related TEAE if any of the events were treatment-related within each system organ class or preferred term, regardless of whether the subject also had an event of the same system organ class or preferred term which was not noted as treatment-related.

Pretreatment AEs are defined as AEs occurring prior to dosing. These events will be presented in the listings only and are not included in the tabular summary of AEs. See Appendix A for handling of partial dates for AEs. In the rare case where it is not possible to assess treatment emergence, the AE will be classified as treatment emergent.

Listings of all AEs, AEs leading to withdrawal from study or permanent discontinuation of study treatment, serious adverse events (SAE), and deaths, if any, will be provided. If deemed relevant (depending on the number of such events observed), summaries of SAEs, AEs leading to discontinuation of study drug, and deaths (with primary cause) will be provided using the same procedure as for TEAE.

4.12.4.3 Pregnancies (as applicable)

A listing of all female subjects of childbearing potential who become pregnant during the study will be provided.

4.12.4.4 Clinical Laboratory Evaluations

All laboratory data will be converted into Système International (SI) units for the analysis.

Protocol-specified clinical laboratory findings (serum chemistry and hematology) will be summarized using descriptive statistics. The statistics will be presented for baseline, each scheduled assessment during the study, and change from baseline to each scheduled assessment.

Based upon laboratory normal ranges, these laboratory test results will be categorized as low (below the lower limit), normal (within normal range), and high (above upper limit). For each treatment, shift tables comparing the distributions of these 3 categories at baseline versus Week 4 (and Week 8) evaluation will be presented for serum chemistry and hematology.

For non-continuous urinalysis variables, data will be summarized by counts and percentages based on the result of test.

Listings of all clinical laboratory data for each subject will be provided with values outside the normal ranges indicated. Scheduled post-baseline laboratory values will be tabulated. A listing of all abnormal laboratory findings will be provided.

The following liver chemistry stopping criteria will be applied to absolute ALT. Occurrences of these abnormalities will be listed separately:

1.   ALT ≥3 × upper limit of normal (ULN)

2.   ALT ≥3 × ULN and bilirubin ≥2 × ULN (>35% direct bilirubin)

3.   ALT ≥3 × ULN and international normalized ratio (INR) >1.5

Criteria (2) and (3) will also be reported as SAE.

4.12.4.5   Vital Signs

Vital signs (temperature, respiratory rate, pulse rate, and systolic and diastolic blood pressure) will be summarized by treatment using descriptive statistics for baseline, each scheduled assessment, and change from baseline to each scheduled assessment. Listings of all vital signs data will be provided.

4.13   Changes from Analyses Planned in Protocol

Electrocardiogram intervals will be listed for only those subjects that had the procedure performed after experiencing an AE.

5.0   SUBJECT DATA LISTINGS

Subject listings of all the data collected in the study database, including data recorded in the eCRFs and any external data transfers (such as clinical laboratory results), will be presented.

6.0   REFERENCES

A Randomized, Open-label Study to Evaluate the Immunogenicity of Anthrax Vaccine Adsorbed Alone or Concomitantly with Raxibacumab (GSK3068483). Clinical study protocol 201436: 21 October 2014.

APPENDIX A. PARTIAL DATE CONVENTIONS

Imputed dates will NOT be presented in the listings.

ALGORITHM FOR TREATMENT EMERGENCE OF ADVERSE EVENTS:

START DATE	STOP DATE	ACTION
Known	Known	If start date < study med start date, then not TEAE If start date >= study med start date, then TEAE
	Partial	If start date < study med start date, then not TEAE If start date >= study med start date, then TEAE
	Missing	If start date < study med start date, then not TEAE If start date >= study med start date, then TEAE
Partial, but known components show that it cannot be on or after study med start date	Known	Not TEAE
	Partial	Not TEAE
	Missing	Not TEAE
Partial, could be on or after study med start date	Known	If stop date < study med start date, then not TEAE If stop date >= study med start date, then TEAE
	Partial	Impute stop date as latest possible date (i.e. last day of month if day unknown or 31st December if day and month are unknown), then: If stop date < study med start date, then not TEAE If stop date >= study med start date, then TEAE
	Missing	Assumed TEAE
Missing	Known	If stop date < study med start date, then not TEAE If stop date >= study med start date, then TEAE

START DATE	STOP DATE	ACTION
	Partial	Impute stop date as latest possible date (i.e. last day of month if day unknown or 31st December if day and month are unknown), then:
		If stop date < study med start date, then not TEAE
		If stop date >= study med start date, then TEAE
	Missing	Assumed TEAE

ALGORITHM FOR PRIOR / CONCOMITANT MEDICATIONS:

START DATE	STOP DATE	ACTION
Known	Known	If stop date < study med start date, assign as prior
		If stop date >= study med start date and start date <= end of treatment, assign as concomitant
		If stop date >= study med start date and start date > end of treatment, assign as post study
	Partial	Impute stop date as latest possible date (i.e. last day of month if day unknown or 31st December if day and month are unknown), then:
		If stop date < study med start date, assign as prior
		If stop date >= study med start date and start date <= end of treatment, assign as concomitant
		If stop date >= study med start date and start date > end of treatment, assign as post treatment
	Missing	If stop date is missing could never be assumed a prior medication
		If start date <= end of treatment, assign as concomitant
		If start date > end of treatment, assign as post treatment
Partial	Known	Impute start date as earliest possible date (i.e. first day of month if day unknown or 1st January if day and month are unknown), then:
		If stop date < study med start date, assign as prior
		If stop date >= study med start date and start date <= end of treatment, assign as concomitant
		If stop date >= study med start date and start date > end of treatment, assign as post treatment

START DATE	STOP DATE	ACTION
	Partial	Impute start date as earliest possible date (i.e. first day of month if day unknown or 1st January if day and month are unknown) and impute stop date as latest possible date (i.e. last day of month if day unknown or 31st December if day and month are unknown), then: If stop date < study med start date, assign as prior If stop date >= study med start date and start date <= end of treatment, assign as concomitant If stop date >= study med start date and start date > end of treatment, assign as post treatment
	Missing	Impute start date as earliest possible date (i.e. first day of month if day unknown or 1st January if day and month are unknown), then: If stop date is missing could never be assumed a prior medication If start date <= end of treatment, assign as concomitant If start date > end of treatment, assign as post treatment
Missing	Known	If stop date < study med start date, assign as prior If stop date >= study med start date, assign as concomitant Cannot be assigned as 'post treatment'
	Partial	Impute stop date as latest possible date (i.e. last day of month if day unknown or 31st December if day and month are unknown), then: If stop date < study med start date, assign as prior If stop date >= study med start date, assign as concomitant Cannot be assigned as 'post treatment'
	Missing	Assign as concomitant